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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SOUTH FLORIDA** [US/US]; Division of Patents and Licensing, 4202 East Fowler Avenue, FAO 126, Tampa, FL 33620-7900 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KERR, William, G.** [US/US]; 4421 W. Watrous Avenue, Tampa, FL 33629 (US). **WANG, Jia-Wang** [CN/US]; 14011 Halstead Court 303, Tampa, FL 33613 (US).

(74) Agents: **LADWIG, Glenn, P.** et al.; Saliwanchik, Lloyd & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606 (US).

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(54) Title: LPS-RESPONSIVE CHS1/BEIGE-LIKE ANCHOR GENE AND THERAPEUTIC APPLICATIONS THEREOF

(57) Abstract: The present invention relates to a novel LPS-responsive and *Beige*-like Anchor gene (*lrba*), variants of the *lrba* gene, fragments of the *lrba* gene, and polypeptides encoded thereby. The subject invention also pertains to *lrba* interfering RNA, and uses thereof. In another aspect, the present invention also includes methods of inhibiting tumor growth in a patient by suppressing *lrba* function.

DESCRIPTION

LPS-RESPONSIVE CHS1/BEIGE-LIKE ANCHOR GENE AND THERAPEUTIC APPLICATIONS THEREOF

The subject invention was made with government support under a research project supported by the National Institutes of Health Grant Nos. RO1 DK54767, R21 AI44333, and PO1 NS27405. The government may have certain rights in this invention.

Cross-Reference to Related Application(s)

This application claims the benefit of provisional patent application Serial No. 60/280,107, filed April 2, 2001, which is hereby incorporated by reference in its entirety, including all nucleic acid sequences, amino acid sequences, figures, tables, and drawings.

Background of the Invention

[0001] Mutations in *chs1/beige* result in a deficiency in intracellular transport of vesicles that leads to a generalized immune deficiency in mouse and man. The function of NK cells, CTL, and granulocytes is impaired by these mutations indicating that polarized trafficking of vesicles is controlled by *chs1/beige* proteins. However, a molecular explanation for this defect has not been identified.

[0002] Lipopolysaccharide (LPS) is a potent inducer of maturation in B cells, monocytes, and dendritic cells that facilitates production of inflammatory cytokines, nitric oxide, and antigen presentation so that these cells can participate in the immune response to bacterial pathogens (Harris, M.R. *et al. Journal of Immunology*, 1984, 133:1202; Tobias, P.S. *et al. Progress in Clinical & Biol. Res.*, 1994, 388:31; Inazawa, M. *et al. Lymphokine Res.*, 1985, 4:343). In an attempt to identify genes involved in the maturation of immune cells, a gene-trapping strategy was developed to identify mammalian genes whose expression is altered by cellular stimuli (Kerr, W.G. *et al. Cold Spring Harbor Symposia on Quantitative Biology*, 1989, 54:767). Several novel LPS-responsive genes were successfully trapped (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947), including the SHIP gene that plays a role in controlling the maturation and proliferation of B cells and

monocytes/macrophages *in vivo* (Huber, M. *et al. Prog. in Biophysics and Molecular Biol.*, 1999, 71:423; Ono, M. *et al. Nature*, 1996, 383:263; Ono, M. *et al. Cell*, 1997, 90:293).

[0003] *Chediak-Higashi* Syndrome (CHS^3) patients suffer from a systematic immune deficiency characterized by a severe immune defect, hypopigmentation, progressive neurologic dysfunction and a bleeding diathesis (Spritz, R.A. *Jour. of Clinical Immun.*, 1998, 18:97). Specific defects in immune cells include defects in T cell cytotoxicity (Abo, T. *et al. Jour. of Clinical Investigation*, 1982, 70:193; Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122), killing by NK cells (Haliotis, T. *et al. Jour. of Exper. Med.*, 1980, 151:1039), defective bactericidal activity and chemotaxis by granulocytes and monocytes (Clark, R.A. and H.R. Kimball *Jour. of Clinical Investigation*, 1971, 50:2645). CHS and *beige* lysosomes also exhibit compartmental missorting of proteins (Takeuchi, K. *et al. Jour. of Exper. Med.*, 1986, 163:665). Other studies have found that *beige* macrophages are defective for class II surface presentation (Faigle, W. *et al. J. Cell Biol.*, 1998, 141:1121; Lem, L. *et al. Jour. of Immun.*, 1999, 162:523) and that T cells in CHS patients are defective for CTLA4 surface expression (Barrat, F.J. *et al. Proc. Natl. Acad. of Sci. USA*, 1999, 96:8645). All cells in *beige* mice and CHS patients bear giant vesicles that cluster around the nucleus. Affected vesicles include lysosomes, platelet dense granules, endosomes, and cytolytic granules. These giant vesicles seem normal in several aspects except for their failure to release their contents, probably resulting from inability of the giant granules to mobilize and/or fuse with the membrane upon stimulation (Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122). However, despite these very provocative findings there still remains no direct evidence that BG(beige)/CHS1 proteins associate with intracellular vesicles and thus a molecular explanation for defective vesicle trafficking and protein missorting in these diseases is still sought.

Brief Summary of the Invention

[0004] The present invention relates to a novel LPS-responsive and *Beige*-like Anchor gene (*lrba*), its transcriptional/translational products, and the targeting of the *lrba* gene for the treatment of cancer. Thus, the present application is directed to the *lrba* gene, variants of the *lrba* gene, fragments of the *lrba* gene, corresponding polypeptides encoding by such nucleotides, and uses thereof. The mouse *lrba* gene is disclosed herein in Figure 1 (SEQ ID NO. 1) and the human *lrba* gene is disclosed herein in Figure 9 (SEQ ID NO. 2). The *lrba* gene is associated with the vesicular system, such as the Golgi complex, lysosomes,

endoplasmic reticu[redacted] plasma membrane and perinuclear ER, [redacted]plays an important role in coupling signal transduction and vesicle trafficking to enable polarized secretion and/or membrane deposition of immune effector molecules. In one aspect, the *lrba* variants of the subject invention include five isoforms of the *lrba* gene, including *lrba*-α, *lrba*-β, *lrba*-δ, *lrba*-γ, and *lrba*-ε. The sequences of the mouse *lrba* cDNAs have been deposited in GENBANK with the following GENBANK accession numbers: lrba-α: AF187731 (SEQ ID NO. 3), lrba-β: AF188506 (SEQ ID NO. 4), lrba-γ: AF188507 (SEQ ID NO. 5).

[0005] The subject invention also relates to cloning and expression vectors containing the *lrba* gene, and fragments and variants thereof, and cells transformed with such vectors.

[0006] In one aspect, the subject invention concerns *lrba* small interfering RNA (siRNA) sequences useful for the treatment of cancer. Preferably, the siRNA duplex is formed by annealing single-stranded RNA sequences (ssRNA) of 5'CCAGCAAAGGUCUUGGCUAdTdT3' (SEQ ID NO. 6) and 5'CAGUCGGGUUUGCGACUGGdTdT3' (SEQ ID NO. 7) from the *lrba* gene.

[0007] In a further aspect, the subject invention concerns methods of inhibiting the growth of tumors in a patient by suppressing *lrba* function. According to the method of the subject invention, suppression of *lrba* function can be carried out at various levels, including the levels of gene transcription, translation, expression, or post-expression. For example, suppression of *lrba* gene expression can be carried out using a variety of modalities known in the art for interfering with the production of a functional product of a target gene. For example, siRNA sequences, such as those described above, can be administered to a patient in need thereof. The siRNA can be produced and administered exogenously, or the siRNA can be inserted into an appropriate vector and the vector can be administered to the patient for production of the siRNA *in vivo*, for example.

[0008] The subject invention also provides methods of detecting the presence of *lrba* nucleic acids, transcriptional products, or polypeptides in samples suspected of containing *lrba* genes, transcriptional products, or polypeptides.

[0009] Another aspect of the subject invention provides kits for detecting the presence of *lrba* genes, *lrba* variants, *lrba* polypeptides, or *lrba* transcriptional products obtained from the polynucleotide sequences.

Brief Description of Drawings

[0010] Figures 1A and 1B show the sequence and structure of the mouse *lrba* gene. Figure 1A shows the predicted full-length amino acid sequence of the *lrba-α* and *lrba-β* (stopped at the boxed "R" with the additional sequence VSAVGSTLFLLLGSSK) and lrba-γ cDNAs (stopped at the boxed "I" with the additional sequence GLPLLSLFAIH). Bold amino acids indicate the BEACH domain (2276-2490) based on alignment with 20 other BEACH domains. Eight WD repeats predicted by an algorithm available at http://bmerc-www.bu.edu/psa/request.htm, are underlined or dotted-underlined. The first three WD repeats are not predicted by other programs but resemble WD repeats and thus are referred to herein as WDL (WD-like) repeats. Two putative protein kinase A RII binding sites are shaded. The sequences of the mouse *lrba* cDNAs have been deposited in GENBANK with the following GENBANK accession numbers: lrba-α: AF187731 (SEQ ID NO. 3), lrba-β: AF188506 (SEQ ID NO. 4), lrba-γ: AF188507 (SEQ ID NO. 5). Figure 1B shows a schematic diagram of mLRBA protein and alignment of the predicted mLRBA protein with its orthologues and some paralogues. The stop sites for the lrba-β and lrba-γ are indicated by dashed lines. The human LRBA protein was predicted from a 9.9 kb "hybrid" cDNA sequence with the first 5' 2577 nucleotides from this work (GENBANK accession numbers AF216648 (SEQ ID NO. 2)) and the rest from the CDC4L partial cDNA sequence (GENBANK accession numbers M83822) (Feuchter, A.E. *et al.* (1992) *Genomics* 13:1237) except one G was added after position 5696 for two reasons: (i) the G base is present in the cDNA sequence (GENBANK accession numbers AF217149); and (ii) this addition extended the CDC4L ORF by an additional 165 AA that had high homology with mLRBA and other proteins shown in this figure. The dLRBA was predicted from the *drosophila melanogaster* genomic sequence (GENBANK accession number AE003433). cLRBA (GENBANK accession number T20719, *Caenorhabditis elegans*), aCDC4L (GENBANK accession number T00867, *Arabidopsis thaliana*), LSVA (GENBANK accession number AAD52096, *Dictyostelium discoideum*), hFAN (GENBANK accession number NP_0035711, *Homo sapiens*), CHS1 (*Chediak-Higashi* Syndrome 1, GENBANK accession number NP_000072, *Homo sapiens*), mBG (GENBANK accession number AAB60778, *Mus musculus*).

[0011] Figures 2A and 2B show the PKA binding sites in LRBA. In Figure 2A, the conservation of hydrophobic amino acids of putative PKA binging sites in mLRBA, hLRBA, dLRBA, and cLRBA are shown by aligning with the known B1 and B2 PKA RII tethering sites (underlined) in DAKAP550 (a partial cDNA sequence for dLRBA) along with other

sequences in these regions. Figure 2B shows the predicted secondary structure of the putative PKA binding sites in mLRBA (mLRBAb1, mLRBAb2). The hydrophobic amino acids on the hydrophobic side of the predicted amphipathic helices are boxed.

[0012] Figure 3 shows the alignment of the C-terminal sequences of mLRBA, hLRBA, dLRBA, CHS1, and hFAN, which include the BEACH domains (in the middle, boxed), 5 WD repeats and the 3 WDL repeats predicted in mLRBA and hLRBA. The predicted SH3, SH2 binding sites and tyrosine kinase recognition sites are also boxed. The C-terminal difference of the three isoforms of the mLRBA, α, β, and γ, are shown here (and Figure 1B in more detail).

[0013] Figures 4A and 4B show that expression of *lrba* is inducible in B cells and macrophages. Figure 4A shows Northern blot hybridization of mRNA from B cell line 70Z/3 and the macrophage cell line J774. Both cell lines were cultured with or without LPS for 20 hours. The poly A^+RNA was purified from these cells, run on a denaturing formaldehyde agarose gell, and transferred to a Hybond-N+ filter. The filter was hybridized with the 2.5 kb probe that corresponds to the coding region of the *lrba* gene including the BEACH and WD domains, as described in the Materials and Methods section. The hybridized filter was exposed to X-ray film for 24 hours. Similar amounts of β-actin mRNA were found in all mRNA tested (Actin panels). Figures 4B and 4C show expression of mRNA of three *lrba* isoforms (α, β, and γ) in B cell lines (Figure 4B) and tissues (Figure 4C). Three isoform-specific primer pairs were used to detect the expression of the three isoforms by RT-PCR, the expected product size of the RT-PCR product for the α form is 1344 bp, for the β form 836 bp, and for the γ form 787 bp. Total RNA is analyzed. Aliquots (10 μl) of the PCR products were resolved on 0.8% agarose gels. Three independent experiments were performed and yielded similar results.

[0014] Figures 5A-5I show subcellular localization of GFP-LRBA fusion proteins revealed by UV-fluorescence microscopy and laser-scan confocal microscopy. Figure 5A shows the RAW 267.4 macrophage cell line (R7) stably transfected with a BEACH-WD-GFP fusion construct. Most cells have diffuse, cytosolic GFP fluorescence, but some cells show vesicle association of the GFP fusion protein. In Figure 5B, the same cell line from Figure 5A was plated on glass-covered plates and stimulated with LPS (100 ng/ml) for 24 hours. Extensive vesicle association of the fusion protein was observed. Figure 5C shows RAW 267.4 macrophages stably transfected with the control vector pEGFP-N2 that were cultured with 100 ng/ml LPS stimulation. No obvious vesicle association of native GFP was

observed. Magnification: 400X. Figure 5D shows part of an R7 macrophage cell, showing GFP fluorescence. Figure 5E shows the same part of an R7 macrophage cell as in Figure 5D, showing acidic lysosomes specifically labeled by LysoTracker Red in living cells. Figure 5F shows lysosome co-localization (white part) of GFP fusion protein by overlapping pictures of Figures 5D and 5E; N = nucleus. Figure 5G shows R7 macrophage cells, showing GFP fluorescence. Figure 5H shows the same R7 macrophage cells as in Figure 5G, showing prominent labeling of the Golgi complex (between the two nuclei) specifically labeled by BODIPY TR ceramide. Other intracellular membranes are weakly labeled. Figure 5I shows Golgi co-localization (white part) of GFP fusion protein by overlapping pictures shown in Figures 5G and 5H. Co-localization was determined by Zeiss LSM 510 software, which allows for a reliability of 99% for actual pixels with both fluorophores. Co-localization mask pixels are converted to white color for clarity. All cells were stimulated with LPS (100 ng/ml) for 24 hours except for Figure 5A.

[0015] Figures 6A-6F show immunoelectron microscopy of LRBA-GFP fusion protein. The LPS-stimulated R7 macrophage cells were fixed and processed for postembedding immunocytochemistry. The cells were dehydrated and embedded in gelatin capsules in LR White resin. Ultrathin sections of LR White embedded cells were collected on nickel grids and immunolabeled with rabbit-anti-GFP followed by labeling with anti-rabbit IgG-gold secondary antibody, and finally stained with uranyl acetate and lead citrate before examination with EM. Figure 6A shows a clathrin-coated pit (endocytic, or coated vesicle) labeled with gold particles (open arrow). This is a vesicle forming on the cell surface. The fact that there is clathrin around this vacuole indicates that it is involved in endocytosis and not exocytosis. Figure 6B shows intense labeling of a primary lysosome (open arrow) and a vesicle on the cell surface (closed arrow). In Figure 6C, the black arrows show ribosomes lining a profile of endoplasmic reticulum (er). There are three gold particles labeling the ER (open arrow). The gray structure next to the ER is a mitochondrion (m), which is not labeled. Figure 6D shows a Golgi region of a cell labeled for GFP. The open arrows show gold particles on a Golgi cisterna. Figure 6E shows labeling of endoplasmic reticulum comprising the perinuclear cisterna (open arrows), and labeling of the plasma membrane of the cell (closed arrows). Figure 6F shows gold particles surrounding a secondary lysosome in a cell (*). At the top of the lysosome is a coated vesicle (closed arrow) fusing with the lysosome. A portion of ER surrounds the bottom of the lysosome, which is also labeled with gold particles (open arrow). Labeling of the perimeter of the

secondary lysosom[redacted]ows routing of GFP from the cell sur[redacted]o the lysosome limiting membrane. In Figures 6A-6F, e = extracellular space; n = nucleus; er = endoplasmic reticulum; g = Golgi; m = mitochondrion; c = cytoplasm. The size of gold particles is 10 nm.

[0016] Figure 7 shows a model of vesicle secretion for WBW protein family using the *lrba* gene as a prototype. Following immune cell activation, the BEACH domain binds to vesicles containing cargo proteins and membrane proteins for secretion or deposition in the plasma membrane. The anchor domain binds to microtubules to move the vesicles to the membrane where the WD domain binds to phosphorylated sequences of membrane receptor complexes to mediate the fusion of the vesicles with the membrane, thus releasing the cargo proteins or depositing membrane proteins on the plasma membrane of immune cells.

[0017] Figure 8 shows a Western blot of a Raw 264.7 macrophage cell line and stably transfected Raw 264.7 cell lines, demonstrating inhibition of apoptosis by LRBA fusion proteins. 586-2 cells were transfected with BEACH-GFP construct; R7 cells were transfected with BEACH-WD-GFP construct; and RGFP cells were transfected with pEGFP vector. The level of both cleaved PARP (poly(ADP-ribose) polymerase and cleaved caspase 3 are higher in control cell lines (Raw 264.7 and RGFP) than in LRBA transfected Raw 264.7 cell lines (586-2 and R7), suggesting LRBA constructs can prevent cells from apoptosis induced by staursporine.

[0018] Figure 9 shows the predicted full-length amino acid sequence and structure of the human *LRBA* gene and its five isoforms. Each isoform is shown by α, β, γ, δ, ε at the right of each C-terminus or the five amino acid insertion(γ). Residues in italic letters indicate isoform-specific sequences. *Asterisk* * = stop codon. Sequences are connected by arrows. The numbers at the right are for the α form. The domains are shaded and named above each domain. Five WD repeats predicted by an algorithm available at http://bmerc-www.bu.edu/psa/request.htm are also shaded or boxed. HSH (helix-sheet-helix); SET: Rich in Serine(S), Glutamic acid(E) and Threnine(T). G peptide has five consecutive glycine. The two potential start codons are boxed. The sequences of the *LRBA* cDNAs have been deposited in GenBank (accession number NM_006726).

[0019] Figure 10 shows secondary structure prediction and alignment of the HSH domain in several WBW proteins. Sequence positions *highlighted in magenta* and *yellow* correspond, respectively, to helices and strands. Sequence positions *highlighted in blue* are potential glycosylation sites. *Squared* positions correspond to conserved residues found in the three WBW protein. The positions of the predicted helical regions of the HSH structure

are indicated as tu[redacted]t the top of the sequences. Sequences h[redacted]g homologues in Figure 9 were analyzed as multiple sequence alignments using the Jpred^2 method (Cuff, J.A. *et al.* (1998) *Bioinformatics* 14:892-893; Cuff J.A. and Barton, G.J. (1999) *Proteins: Structure, Function and Genetics* 34:508-519; Cuff, J.A. and Barton, G.J. (1999) *Proteins: Structure, Function and Genetics* 40:502-511). Several sequences that, after a first prediction run, were found to have more than 25% homology in one of the three conserved helical regions were reprocessed together as a multiple sequence alignment using Jpred^2 to refine the prediction of that particular region. Secondary structure predictions were performed by the Jpred^2 method. *Rectangles* indicate -helices and *arrows* indicate -strands. HSH (helix-sheet-helix) domain: Several WBW proteins have a high homology and a common predicted protein secondary structure (HSH structure) over an 100 amino acid stretch near their N-terminus, as shown in Figure 10. Because the HSH domain exists in evolutionarily very distant species (Dictyostelium is a cellular slime mold, more ancient than yeast), it may have important function in a cell's life. SET domain: rich in serine (S, 13.70%), glutamic acid (E, 13.40%) and threonine (T, 9.03%). Its function is still unknown. This domain is very hydrophobic and has a very high antigenic index. PI is 3.96.

[0020] Figure 11 shows the genomic structure of the human *LRBA* gene. The gene contains 59 exons, which span more than 700 kb. The exon/intron structure of the *LRBA* gene is mapped to the corresponding cDNA regions encoded by each exon. Location and size of exons and introns are drawn to scale (GenBank accession number NM_006726).

[0021] Figure 12 shows a molecular phylogenic tree of the amino acid sequences of WBW genes from various species. The tree was constructed by the neighbor joining method, based on sequence alignment conducted by CLUSTALX software using either whole length sequence or only the BEACH domain, which gave very similar results. This indicates that the BEACH domain is co-evolving with the rest sequence of the gene and, as the whole sequences of some WBW genes are still unavailable (moreover, the length of the BEACH domain is relatively consistent (around 278 amino acids)), using the BEACH domain seems more reasonable. Thus, Figure 12 is based on the BEACH domain. All the sequences are from GeneBank. The numbers in parenthesis are GI numbers.

[0022] Figure 13 shows alternative splicing of the human *LRBA* gene. The solid or gray box indicates coding exon, and the hatched box indicates UTR (untranslated region). The top numbers indicate exons in the main form (constitutive isoform versus alternative isoform) of human *lrba*, while the bottom numbers indicate alternative splicing isoforms of

the human *LRBA* [redacted] The single Greek letters denote the five [redacted]orms. The *LRBA*δ has a 310 bp Alu sequence at its poly(A) tail. 5'-1, 3'-1 and 3'-2 indicate 5' end and 3' end splicing, while I-1 and I-2 represent internal splicing. 5'-1 splicing gives alternative transcription start site and suggests alternative promoter for human *LRBA* gene. The internal splicing I-1 interrupts the coding sequence of *LRBA*, splitting *LRBA* into two open reading frames (ORF), and thus alternative potential start codon ATG (the meaning of this splicing is further described and discussed later). Another internal alternative splicing I-2 is a 15 bp sequence in frame with the main ORF, inserting a YLLLQ insertion into the human *LRBA* protein (noting that the l and w are hydrophobic amino acids). AATAAA indicate a polyadenine signal. 3'-1 and 3'-2 splicing generate two additional different 3' UTR tails for human *LRBA* gene. The isoform identification was conducted by using the following cultured cells and tissues: (1.) human pre-B (6417) cells; (2.) human Raji B cells; (3.) 293 cells; (4.) human MCF7 breast cancer cells; (5.) human HTB4 lung cancer; (6.) human H322 human lung cancer; (7.) human A539 human lung cancer; (8.) human lung carcinoma; (9.) human lung carcinoma adjacent tissue; (10.) human B-cell lymphoma; (11.) human B-cell lymphoma; and (12.) normal adjacent tissue (3 pairs of tumor tissue and adjacent tissue of human prostate).

[0023] Figures 14A and 14B show results of a 5'RACE (rapid amplification of cDNA end) procedure and 3'RACE procedure, respectively, conducted on the human *lrba* gene. In Figure 14B, the lower band contains an AluSx repeat sequence 312 bp long. RNAs were from: (1.) pre-B (6417); (2.) Raji B cells; (3.) 293 cells; (4.) MCF7 breast cells; (5.) HTB4 lung cancer; (6.) H322 human lung cancer; (7.) A539 human lung cancer; (8.) human lung carcinoma; (9.) human lung carcinoma adjacent tissue; (10.) B-cell lymphoma; (11.) B-cell lymphoma; and (12.) normal adjacent tissue.

[0024] Figure 15 shows the 5' end of the human *lrba*ε isoform with a long 5' UTR. There are four small ORFs before the major ORF of the human *lrba* gene. The longest small ORF encodes the first 73 amino acids of the h*lrba* protein and is in frame with the major ORF, though there are four in-frame stop codons and 6 out-of-frame stop codons, in between which would prevent potential read-through that makes a fusion protein. The other three ORFs encode 20 amino acids, 18 amino acids, and 15 amino acids, respectively. The partial major coding sequence is in bold. The amino acid sequence in italics is present in the main form of the *LRBA* gene but absent in the delta form of the *LRBA* gene. The grey shaded sequence is the extra exon that has interrupted the *LRBA* sequence.

[0025] Fig. 16 shows the predicted secondary structure of RNA sequence between the two ORF of human *lrbaδ*. The free energy for the structure is -40.29 kcal/mol. This suggests a potential IRES (internal ribosome entry signal). There is no homologous sequence between IRES, however they all have complex secondary structure like long stem structure.

[0026] Figure 17 shows the promoter and part of the 5' cDNA sequence of the human *lrba* gene. Transcription start sites as determined by 5'RACE procedure are indicated by arrows. Sequence for a CpG island is in bold. The DNA consensus binding motifs for various transcription factors shown in the region -1561 to +1 were identified using the TFSEARCH (version 1.3) software (Yukata Akiyama (Kyoto Univ.)), the first nucleotide of the most 5' cDNA denoted as 1. The initiator methionine is in bold. The transcription binding sites are shaded, boxed, or underlined. The genomic sequences have GenBank accession number AC104796.

[0027] Figures 18A and 18B show RT-PCR of human prostate tumor tissue and adjacent normal tissue, demonstrating that LRBA expression is increased in human prostate cancer relative to matched normal tissue controls. Figure 18A shows RT-PCR detection of human LRBA mRNA. Figure 18B shows RT-PCR detection of human β-Actin mRNA to control for the amounts of mRNA present. The PCR cycle parameters were as follows: 94° C for 30 seconds, 68° C for 30 seconds, 72° C for 1 minute, 25 cycles. The sources from the matched samples are (from left to right) 1, 3, and 5: prostate adenocarcinoma tissue; 2, 4, and 6: normal prostate tissue. Samples 1 & 2, 3 & 4, and 5 & 6, are matched pairs from three different prostate cancer patients.

[0028] Figure 19 shows growth inhibition of human breast cancer cells by expression of a dominant negative human LRBA mutant. MCF7 human breast cancer cells were seeded ($1x10^4$/well) into a 96-well plate. On the second day, cells were infected with various titers of a recombinant adenovirus that contains a dominant negative LRBA mutant, in the presence or absence of doxycycline. The BW-GFP mutant comprises the BEACH and WD domains of LRBA fused to GFP. The adenoviral vector has a tetracycline-responsive promoter that is repressed in the presence of doxycycline and, thus, the BW-GFP mutant is expressed in the absence of doxycycline. Three days post-infection, the cells were labeled with ^{3}H-thymidine, the cells harvested and CPM incorporated into high molecular weight DNA counted as a measure of cell proliferation (DNA synthesis).

[0029] Figures 20A-20C show the knock-down of *Lrba* expression by LRBA siRNA treatment and death of cancer cells. HeLa cells were plated 2×10^4 cells/well of a 24-well

dish. The next da[redacted]lls were transfected as indicated or wer[redacted] untreated (Blank). The cells were photographed 72 hours after transfected and the wells harvested for cell counting. HeLa cells (human adenocarcinoma) transfected with Lrba siRNA and lipofectamine (Figure 20A) or mock transfected with H_2O and lipofectamine (Figure 20B). Magnification is 400x. Note the presence of apoptotic or necrotic cell bodies as well as the spindly, stressed morphology of the remaining adherent cells in the siRNA *Lrba*-treated well. Figure 20C shows absolute cell numbers recovered as determined by Coulter Counter. Students' T-test: $P<0.0006$ for mock versus Lrba siRNA; $P<0.0036$ for Blank versus Lrba siRNA; $P<0.2271$ for mock versus blank. The siRNA treated cultures show a statistically significant decrease in cell number as compared to either mock or blank cultures, but there is no significant difference in the number of cells recovered from the mock and blank cultures. The RNA sequences that were annealed to make the *Lrba* siRNA were: *Lrba* sense-strand: 5'CCAGCAAAGGUCUUGGCUAdTdT3' (SEQ ID NO. 6); *Lrba* antisense-strand: 5'UAGCCAAGACCUUUGCUGGdTdT3' (SEQ ID NO. 8).

[0030] Figures 21A-21D show silencing of the *Lrba* gene in MCF7 human breast cancer cells and MCF10A human breast normal cells by two pairs of Lrba siRNA (siRNA1 and siRNA2), demonstrating that *Lrba* siRNAs selectively kill human breast cancer cells but not normal cells. MCF7 cells (Figure 21A-21C) and MCF10A cells (Figure 21D) were seeded at 2×10^4 cells per well in 24-well plates. One day later, the cells were transfected with *Lrba* siRNAs or with scramble siRNA as a negative control using oligofectamine. After 72 hours of siRNA treatment, the photos (Figure 21A, MCF7 transfected with siRNA1; Figure 21B, MCF7 transfected with scramble siRNA negative control; magnification 400x) were taken and the cell numbers were counted by a Coulter counter. T-test: Figure 21C (MCF7), $P=0.0009$ for scramble negative control versus siRNA1; $P=0.0005$ for scramble negative control 1 versus *Lrba* siRNA2; $P=0.004$ for siRNA1 versus siRNA2. Figure 21D (MCF10A), $P=0.4070$ for scramble negative control versus siRNA1; $P=0.9456$ for scramble negative control 1 versus *Lrba* siRNA2; $P=0.0514$ for siRNA1 versus siRNA2. The siRNA sequences: siRNA1: CCAGCAAAGGUCUUGGCUAdTdT (SEQ ID NO. 6); siRNA2: GGGCACUCUUUCUGUCACCdTdT (SEQ ID NO. 9); scramble negative control: CAGUCGGGUUUGCGACUGGdTdT (SEQ ID NO. 7).

[0031] Figures 22A-22F show upregulation of *lrba* promoter activity by p53 and E2F transcription factors. The GFP reporter (GFP gene is placed downstream of the lrba gene promoter, designated pLP-GFP) construct was transfected into 293T cells with or without

p53 or E2F wild t[redacted]vector. The pictures were taken one d[redacted]ter transfection. FACS analysis was carried out 60 hours after transfection. The results show that there is 0.7% GFP positive cells in pLP-GFP only (Figures 22A and 22D), 6.88% in pLP-GFP + p53 vector (Figure 22B and 22E), 2.06% in pLP-GFP + pE2F1 vector (Figure 22C and 22F), suggesting that only a small fraction of cells have detectable lrba promoter activity, p53 and E2F can induce the lrba promoter activity to 9.8, 3-fold respectively. p53 and E2F are important cell cycle and apoptosis mediators. All or most tumors can be characterized as being defective in p53 function.

Detailed Disclosure of the Invention

[0032] The subject invention concerns a method of inhibiting cancerous tumor growth in a patient by suppressing *lrba* function. Preferably, the method comprises suppressing the functional expression of the *lrba* gene. Various methods known in the art for suppressing the functional expression of a gene can be utilized to carry out this method of the subject invention. The *lrba* gene can be disrupted partially (*e.g.*, a leaky mutation), resulting, for example, in reduced expression, or the *lrba* gene can be fully disrupted (*e.g.*, complete gene ablation). Such mutations can include, for example, point mutations, such as transitions or transversions, or insertions and/or deletions, and the mutation can occur in the coding region encoding *lrba* or merely in its regulatory sequences. According to the method of the subject invention, functional expression of the *lrba* gene can be suppressed at any level. In another aspect, the subject invention includes methods of disrupting expression of the *lrba* gene *in vivo* or *in vitro*.

[0033] Using the method of the subject invention, *lrba* function is suppressed, which causes inhibition of tumor growth. Preferably, the suppression of *lrba* function results in death of tumor cells. More preferably, *lrba* function is suppressed to an extent that normal (non-cancerous) cells are not killed.

[0034] Various means for suppression of *lrba* function can be utilized according to the method of the subject invention. For example, suppression of *lrba* function can be carried by administration of an agent that directly or indirectly causes suppression of *lrba* function. Agents suitable for the method of the subject invention include nucleic acids, such as a genetic construct or other genetic means for directing expression of an antagonist of *lrba* function. Nucleic acid molecules suitable for the method of the invention include, for example, anti-sense polynucleotides, or other polynucleotides that bind to *lrba* mRNA, for

example. Preferably, the nucleic acid molecules administered to the patient are those disclosed herein. Other agents that can be utilized to carry out suppression of *lrba* function include, for example, peptidomimetics, ribozymes, and RNA aptamers.

[0035] According to the method of the subject invention, polypeptides can be administered to a patient in order to suppress *lrba* function and inhibit tumor growth. Preferably, the polypeptides utilized are those disclosed herein. More preferably, the polypeptides comprise fragments of the full-length *lrba* amino acid sequence (including fragments of full-length amino acid sequences of *lrba* orthologs). Most preferably, the polypeptides comprise amino acid sequences corresponding to the BEACH domain, WD domain, or BEACH and WD domains, of the *lrba* gene (including *lrba* gene orthologs). Various means for delivering polypeptides to a cell can be utilized to carry out the method of the subject invention. For example, protein transduction domains (PTDs) can be fused to the polypeptide, producing a fusion polypeptide, in which the PTDs are capable of transducing the polypeptide cargo across the plasma membrane (Wadia, J.S. and Dowdy, S.F., *Curr. Opin. Biotechnol.*, 2002, 13(1)52-56). Examples of PTDs include the Drosophila homeotic transcription protein antennapedia (Antp), the herpes simples virus structural protein VP22, and the human immuno-deficiency virus 1 (HIV-1) transcriptional activator Tat protein.

[0036] According to the method of tumor inhibition of the subject invention, recombinant cells can be administered to a patient, wherein the recombinant cells have been genetically modified to express an lrba gene product, such as a portion of the amino acid sequences set forth in Figure 1 or Figure 9, or variants thereof.

[0037] The method of tumor inhibition of the subject invention can be used to treat patient suffering from cancer or as a cancer preventative. The method of tumor inhibition of the subject invention can be used to treat patients suffering from a variety of cancers including, but not limited, to cancer of the breast, prostate, melanoma, chronic myelogenous leukemia, cervical cancer, adenocarcinoma, lymphoblastic leukemia, colorectal cancer, and lung carcinoma.

[0038] In another aspect, the subject invention provides isolated and/or purified nucleotide sequences comprising: (i) a polynucleotide sequence encoding the amino acid sequence set forth in Figure 1 or Figure 9, or a complement thereof; (ii) a polynucleotide sequence having at least about 20% to 99.99% identity to the polynucleotide sequence of (i); (iii) a polynucleotide encoding a fragment of the amino acid sequence shown in Figure 1 or

Figure 9; or (iv) [redacted] interfering RNA sequence correspond[redacted] to the transcript of the polynucleotide set forth in Figure 1 or Figure 9, or a fragment of the transcript.

[0039] Nucleotide, polynucleotide, or nucleic acid sequences(s) are understood to mean, according to the present invention, either a double-stranded DNA, a single-stranded DNA, or products of transcription of the said DNAs (*e.g.*, RNA molecules). It should also be understood that the present invention does not relate to the genomic nucleotide sequences encoding *lrba* in their natural/native environment or natural/native state. The nucleic acid, polynucleotide, or nucleotide sequences of the invention have been isolated, purified (or partially purified), by separation methods including, but not limited to, ion-exchange chromatography, molecular size exclusion chromatography, affinity chromatography, or by genetic engineering methods such as amplification, cloning or subcloning.

[0040] Optionally, the polynucleotide sequences of the instant invention can also contain one or more polynucleotides encoding heterologous polypeptide sequences (*e.g.*, tags that facilitate purification of the polypeptides of the invention (see, for example, U.S. Patent No. 6,342,362, hereby incorporated by reference in its entirety; Altendorf *et al.* [1999-WWW, 2000] "Structure and Function of the F_o Complex of the ATP Synthase from *Escherichia Coli*," *J. of Experimental* Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] "Recombinant Protein Expression in *Escherichia coli*," Biotechnology 10:411-21, Elsevier Science Ltd.; Eihauer *et al.* [2001] "The FLAG™ Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins," *J. Biochem Biophys Methods* 49:455-65; Jones *et al.* [1995] *J. Chromatography* 707:3-22; Jones *et al.* [1995] "Current Trends in Molecular Recognition and Bioseparation," *J. of Chromatography A.* 707:3-22, Elsevier Science B.V.; Margolin [2000] "Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells," *Methods* 20:62-72, Academic Press; Puig *et al.* [2001] "The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification," *Methods* 24:218-29, Academic Press; Sassenfeld [1990] "Engineering Proteins for Purification," TibTech 8:88-93; Sheibani [1999] "Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins," *Prep. Biochem. & Biotechnol.* 29(1):77-90, Marcel Dekker, Inc.; Skerra *et al.* [1999] "Applications of a Peptide Ligand for Streptavidin: the *Strep*-tag", *Biomolecular Engineering* 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," *The* Scientist 12(22):20; Smyth *et al.* [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular

Matrix Proteins Ca[redacted]g the Strep II Tag", *Methods in Molecu[redacted]iology*, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and Purification Systems," *The Scientist* 11(17):20, each of which is hereby incorporated by reference in their entireties), or commercially available tags from vendors such as such as STRATAGENE (La Jolla, CA), NOVAGEN (Madison, WI), QIAGEN, Inc., (Valencia, CA), or INVITROGEN (San Diego, CA).

[0041] Other aspects of the invention provide vectors containing one or more of the polynucleotides of the invention. The vectors can be vaccine, replication, or amplification vectors. In some embodiments of this aspect of the invention, the polynucleotides are operably associated with regulatory elements capable of causing the expression of the polynucleotide sequences. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations of the aforementioned vector sources, such as those derived from plasmid and bacteriophage genetic elements (*e.g.*, cosmids and phagemids).

[0042] As indicated above, vectors of this invention can also comprise elements necessary to provide for the expression and/or the secretion of a polypeptide encoded by the nucleotide sequences of the invention in a given host cell. The vector can contain one or more elements selected from the group consisting of a promoter, signals for initiation of translation, signals for termination of translation, and appropriate regions for regulation of transcription. In certain embodiments, the vectors can be stably maintained in the host cell and can, optionally, contain signal sequences directing the secretion of translated protein. Other embodiments provide vectors that are not stable in transformed host cells. Vectors can integrate into the host genome or be autonomously-replicating vectors.

[0043] In a specific embodiment, a vector comprises a promoter operably linked to a protein or peptide-encoding nucleic acid sequence, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). Non-limiting exemplary vectors for the expression of the polypeptides of the invention include pBr-type vectors, pET-type plasmid vectors (Promega), pBAD plasmid vectors (Invitrogen) or those provided in the examples below. Furthermore, vectors according to the invention are useful

for transforming h[redacted]ells for the cloning or expression of the [redacted]leotide sequences of the invention.

[0044] Promoters which may be used to control expression include, but are not limited to, the CMV promoter, the SV40 early promoter region (Bernoist and Chambon [1981] *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.* [1980] *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.* [1981] *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.* [1982] *Nature* 296:39-42); prokaryotic vectors containing promoters such as the β-lactamase promoter (Villa-Kamaroff, *et al.* [1978] *Proc. Natl. Acad. Sci. USA* 75:3727-3731), or the *tac* promoter (DeBoer, *et al.* [1983] *Proc. Natl. Acad. Sci. USA* 80:21-25); see also, "Useful Proteins from Recombinant Bacteria" in *Scientific American*, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.* [1983] *Nature* 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.* [1981] *Nucl. Acids Res.* 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.* [1984] *Nature* 310:115-120); promoter elements from yeast or fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, and/or the alkaline phosphatase promoter.

[0045] The subject invention also provides for "homologous" or "modified" nucleotide sequences. Modified nucleic acid sequences will be understood to mean any nucleotide sequence obtained by mutagenesis according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the normal sequences. For example, mutations in the regulatory and/or promoter sequences for the expression of a polypeptide that result in a modification of the level of expression of a polypeptide according to the invention provide for a "modified nucleotide sequence". Likewise, substitutions, deletions, or additions of nucleic acid to the polynucleotides of the invention provide for "homologous" or "modified" nucleotide sequences. In various embodiments, "homologous" or "modified" nucleic acid sequences have substantially the same biological or serological activity as the native (naturally occurring) LRBA polypeptides. A "homologous" or "modified" nucleotide sequence will also be understood to mean a splice variant of the polynucleotides of the instant invention or any nucleotide sequence encoding a "modified polypeptide" as defined below.

[0046] A h[redacted]ogous nucleotide sequence, for the purp[redacted] of the present invention, encompasses a nucleotide sequence having a percentage identity with the bases of the nucleotide sequences of between at least (or at least about) 20.00% to 99.99% (inclusive). The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire sequence length.

[0047] In various embodiments, homologous sequences exhibiting a percentage identity with the bases of the nucleotide sequences of the present invention can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polynucleotide sequences of the instant invention.

[0048] Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman [1988] *Proc. Natl. Acad. Sci. USA* 85(8):2444-2448; Altschul *et al.* [1990] *J. Mol. Biol.* 215(3):403-410; Thompson *et al.* [1994] *Nucleic Acids Res.* 22(2):4673-4680; Higgins *et al.* [1996] *Methods Enzymol.* 266:383-402; Altschul *et al.* [1990] *J. Mol. Biol.* 215(3):403-410; Altschul *et al.* [1993] *Nature Genetics* 3:266-272).

[0049] The subject invention also provides nucleotide sequences complementary to any of the polynucleotide sequences disclosed herein. Thus, the invention is understood to include any DNA whose nucleotides are complementary to those of the sequence of the invention, and whose orientation is reversed (*e.g.*, an antisense sequence).

[0050] The present invention further provides fragments of the polynucleotide sequences provided herein. Representative fragments of the polynucleotide sequences according to the invention will be understood to mean any nucleotide fragment having at least 8 or 9 successive nucleotides, preferably at least 12 successive nucleotides, and still more preferably at least 15 or at least 20 successive nucleotides of the sequence from which it is derived. The upper limit for such fragments is the total number of polynucleotides found in the full-length sequence (or, in certain embodiments, of the full length open reading frame (ORF) identified herein). It is understood that such fragments refer only to portions of the

disclosed polynucleotide sequences that are not listed in a publicly available database or prior art references. However, it should be understood that with respect to the method for inhibiting tumor growth of the subject invention, disclosed nucleotides (and polypeptides encoded by such nucleotides) that are listed in a publicly available database or prior art reference can also be utilized. For example, nucleotide sequences that are *lrba* orthologs, or fragments thereof, which have been previously identified, can be utilized to carry out the method for inhibiting tumor growth of the subject invention. Thus sequences from the *drosophila melanogaster* genomic sequence (GENBANK accession number AE003433), cLRBA (GENBANK accession number T20719, *Caenorhabditis elegans*), aCDC4L (GENBANK accession number T00867, *Arabidopsis thaliana*), LSVA (GENBANK accession number AAD52096, *Dictyostelium discoideum*), hFAN (GENBANK accession number NP_0035711, *Homo sapiens*), CHS1 (*Chediak-Higashi* Syndrome 1, GENBANK accession number NP_000072, *Homo sapiens*), or mBG (GENBANK accession number AAB60778, *Mus musculus*) can be utilized to carry out the method of tumor growth inhibition of the subject invention.

[0051] In other embodiments, fragments contain from one nucleotide less than the full length polynucleotide sequence (1249 nucleotides) to fragments comprising up to, and including 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, . . . and up to, for example, 1,245 consecutive nucleotides of a particular sequence disclosed herein.

[0052] Yet other embodiments provide fragments (or detection probes) comprising nucleotides within the *lrba* cDNA sequence, such as the human *lrba* cDNA sequence (GenBank accession number NM_006726), including 245 to 458 (G-peptide), 488 to 1424 (HSH domain), 2573-2627 (siRNA1) (SEQ ID NO. 6), 3179 to 4148 (SET domain), 4301 to 4505 (PKA RII binding sites), 6347 to 6749 (WDL repeats), 6878 to 7709 (BEACH domain), 8018 to 8831 (WD repeats).

[0053] Among these representative fragments, those capable of hybridizing under stringent conditions with a nucleotide sequence according to the invention are preferred. Conditions of high or intermediate stringency are provided *infra* and are chosen to allow for hybridization between two complementary DNA fragments. Hybridization conditions for a polynucleotide of about 300 bases in size will be adapted by persons skilled in the art for

larger- or smaller- oligonucleotides, according to methods known in the art (see, for example, Sambrook *et al.* [1989]).

[0054] The subject invention also provides detection probes (*e.g.*, fragments of the disclosed polynucleotide sequences) for hybridization with a target sequence or an amplicon generated from the target sequence. Such a detection probe will advantageously have as sequence a sequence of at least 9, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides. Alternatively, detection probes can comprise 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, . . . and up to, for example, 1245 consecutive nucleotides of the disclosed nucleic acids. The detection probes can also be used as labeled probe or primer in the subject invention. Labeled probes or primers are labeled with a radioactive compound or with another type of label. Alternatively, non-labeled nucleotide sequences may be used directly as probes or primers; however, the sequences are generally labeled with a radioactive element (^{32}P, ^{35}S, ^{3}H, ^{125}I) or with a molecule such as biotin, acetylaminofluorene, digoxigenin, 5-bromo-deoxyuridine, or fluorescein to provide probes that can be used in numerous applications.

[0055] The nucleotide sequences according to the invention may also be used in analytical systems, such as DNA chips. DNA chips and their uses are well known in the art and (see for example, U.S. Patent Nos. 5,561,071; 5,753,439; 6,214,545; Schena *et al.* [1996] *BioEssays* 18:427-431; Bianchi *et al.* [1997] *Clin. Diagn. Virol.* 8:199-208; each of which is hereby incorporated by reference in their entireties) and/or are provided by commercial vendors such as AFFYMETRIX, Inc. (Santa Clara, CA).

[0056] Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak [1987] *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[0057] By way of example, hybridization of immobilized DNA on Southern blots with ^{32}P-labeled gene-specific probes can be performed by standard methods (Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New

York). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25°C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz *et al.* [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

T_m=81.5°C+16.6 Log[Na+]+0.41(%G+C)-0.61(% formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash);

(2) once at T_m-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[0058] For oligonucleotide probes, hybridization can be carried out overnight at 10-20°C below the melting temperature (T_m) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes can be determined by the following formula:

T_m (°C)=2(number T/A base pairs) + 4(number G/C base pairs) (Suggs *et al.* [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes can be carried out as follows:

(1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash;

2) once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[0059] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42°C
Moderate:	0.2X or 1X SSPE, 65°C
High:	0.1X SSPE, 65°C.

[0060] By [redacted] of another non-limiting example, proced[redacted] using conditions of high stringency can also be performed as follows: Pre-hybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C, the preferred hybridization temperature, in pre-hybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Alternatively, the hybridization step can be performed at 65°C in the presence of SSC buffer, 1 x SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1X SSC at 50°C for 45 min. Alternatively, filter washes can be performed in a solution containing 2 x SSC and 0.1% SDS, or 0.5 x SSC and 0.1% SDS, or 0.1 x SSC and 0.1% SDS at 68°C for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. Other conditions of high stringency which may be used are well known in the art (see, for example, Sambrook *et al.* [1989] *Molecular Cloning, A Laboratory Manual, Second Edition*, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.* [1989] *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., each incorporated herein in its entirety).

[0061] A further non-limiting example of procedures using conditions of intermediate stringency are as follows: Filters containing DNA are pre-hybridized, and then hybridized at a temperature of 60°C in the presence of a 5 x SSC buffer and labeled probe. Subsequently, filters washes are performed in a solution containing 2x SSC at 50°C and the hybridized probes are detectable by autoradiography. Other conditions of intermediate stringency which may be used are well known in the art (see, for example, Sambrook *et al.* [1989] *Molecular Cloning, A Laboratory Manual, Second Edition*, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.* [1989] *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., each of which is incorporated herein in its entirety).

[0062] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be

produced in a given polynucleotide sequence in many ways, and some methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

[0063] It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.* [1983] *J. Biol. Chem.* 258:13006-13512. The nucleic acid sequences of the subject invention can also be used as molecular weight markers in nucleic acid analysis procedures.

[0064] The invention also provides host cells transformed by a polynucleotide according to the invention and the production of LRBA (or LRBA ortholog) polypeptides by the transformed host cells. In some embodiments, transformed cells comprise an expression vector containing LRBA, or LRBA ortholog, polynucleotide sequences. Other embodiments provide for host cells transformed with nucleic acids. Yet other embodiments provide transformed cells comprising an expression vector containing fragments of *lrba*, or *lrba* ortholog, polynucleotide sequences. Transformed host cells according to the invention are cultured under conditions allowing the replication and/or the expression of the nucleotide sequences of the invention. Expressed polypeptides are recovered from culture media and purified, for further use, according to methods known in the art.

[0065] The host cell may be chosen from eukaryotic or prokaryotic systems, for example bacterial cells (Gram negative or Gram positive), yeast cells, animal cells, plant cells, and/or insect cells using baculovirus vectors. In some embodiments, the host cell for expression of the polypeptides include, and are not limited to, those taught in U.S. Patent Nos. 6,319,691; 6,277,375; 5,643,570; 5,565,335; Unger [1997] *The Scientist* 11(17):20; or Smith [1998] *The Scientist* 12(22):20, each of which is incorporated by reference in its entirety, including all references cited within each respective patent or reference. Other exemplary, and non-limiting, host cells include *Staphylococcus* spp., *Enterococcus* spp., *E. coli*, and *Bacillus subtilis*; fungal cells, such as *Streptomyces* spp., *Aspergillus* spp., *S. cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*, *Hansela polymorpha*, *Kluveromyces lactis*, and *Yarrowia lipolytica*; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells. A great variety of expression systems can be used to produce the polypeptides of the invention and polynucleotides can be modified according to

methods known in the art to provide optimal codon usage for expression in a particular expression system.

[0066] Furthermore, a host cell strain may be chosen that modulates the expression of the inserted sequences, modifies the gene product, and/or processes the gene product in the specific fashion. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product whereas expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to provide "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

[0067] Nucleic acids and/or vectors can be introduced into host cells by well-known methods, such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection (see, for example, Sambrook *et al.* [1989] *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

[0068] The subject invention also provides for the expression of a polypeptide, derivative, or a variant (*e.g.*, a splice variant) encoded by a polynucleotide sequence disclosed herein. Alternatively, the invention provides for the expression of a polypeptide fragment obtained from a polypeptide, derivative, or a variant encoded by a polynucleotide fragment derived from the polynucleotide sequences disclosed herein. In either embodiment, the disclosed sequences can be regulated by a second nucleic acid sequence so that the polypeptide or fragment is expressed in a host transformed with a recombinant DNA molecule according to the subject invention. For example, expression of a protein or peptide may be controlled by any promoter/enhancer element known in the art.

[0069] The subject invention also provides nucleic acid based methods for the identification of the presence of the *lrba* gene, or orthologs thereof, in a sample. These methods can utilize the nucleic acids of the subject invention and are well known to those

skilled in the art, see, for example, Sambrook *et al.* [19[redacted]or Abbaszadega [2001] "Advanced Detection of Viruses and Protozoan Parasites in Water," *Reviews in Biology and Biotechnology*, 1(2):21-26). Among the techniques useful in such methods are enzymatic gene amplification (or PCR), Southern blots, Northern blots, or other techniques utilizing nucleic acid hybridization for the identification of polynucleotide sequences in a sample. The nucleic acids can be used to screen individuals for cancers, tumors, or malignancies associated with dysregulation of the *lrba* gene or its transcriptional products.

[0070] The subject invention also provides polypeptides encoded by nucleotide sequences of the invention. The subject invention also provides fragments of at least 5 amino acids of a polypeptide encoded by the polynucleotides of the instant invention.

[0071] In the context of the instant invention, the terms polypeptide, peptide and protein are used interchangeably. Likewise, the terms variant and homologous are also used interchangeably. It should be understood that the invention does not relate to the polypeptides in natural form or native environment. Peptides and polypeptides according to the invention have been isolated or obtained by purification from natural sources (or their native environment), chemically synthesized, or obtained from host cells prepared by genetic manipulation (*e.g.*, the polypeptides, or fragments thereof, are recombinantly produced by host cells). Polypeptides according to the instant invention may also contain non-natural amino acids, as will be described below.

[0072] "Variant" or "homologous" polypeptides will be understood to designate the polypeptides containing, in relation to the native polypeptide, modifications such as deletion, addition, or substitution of at least one amino acid, truncation, extension, or the addition of chimeric heterologous polypeptides. Optionally, "variant" or "homologous" polypeptides can contain a mutation or post-translational modifications. Among the "variant" or "homologous" polypeptides, those whose amino acid sequence exhibits 20.00% to 99.99% (inclusive) identity to the native polypeptide sequence are preferred. The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 50.00% and, up to, including 99.99%. These percentages are purely statistical and differences between two polypeptide sequences can be distributed randomly and over the entire sequence length.

[0073] "Variant" or "homologous" polypeptide sequences exhibiting a percentage identity with the polypeptides of the present invention can, alternatively, have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,

49, 50, 51, 52, 53, [redacted]5, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, [redacted]7, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 91, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polypeptide sequences of the instant invention. The expression equivalent amino acid is intended here to designate any amino acid capable of being substituted for one of the amino acids in the basic structure without, however, essentially modifying the biological activities of the corresponding peptides and as provided below.

[0074] By way of example, amino acid substitutions can be carried out without resulting in a substantial modification of the biological activity of the corresponding modified polypeptides; for example, the replacement of leucine with valine or isoleucine; aspartic acid with glutamic acid; glutamine with asparagine; arginine with lysine; and the reverse substitutions can be performed without substantial modification of the biological activity of the polypeptides.

[0075] In other embodiments, homologous polypeptides according to the subject invention also include various splice variants identified within the *lrba* coding sequence.

[0076] The subject invention also provides biologically active fragments of a polypeptide according to the invention and includes those peptides capable of eliciting an immune response. The immune response can provide components (either antibodies or components of the cellular immune response (*e.g.*, B-cells, helper, cytotoxic, and/or suppressor T-cells) reactive with the biologically active fragment of a polypeptide, the intact, full length, unmodified polypeptide disclosed herein, or both the biologically active fragment of a polypeptide and the intact, full length, unmodified polypeptides disclosed herein. Biologically active fragments according to the invention comprise from five (5) amino acids to one amino acid less than the full length of any polypeptide sequence provided herein. Alternatively, fragments comprising 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, . . . and up to 2845 consecutive amino acids of a disclosed polypeptide sequence are provided herein.

[0077] Fragments, as described herein, can be obtained by cleaving the polypeptides of the invention with a proteolytic enzyme (such as trypsin, chymotrypsin, or collagenase) or with a chemical reagent, such as cyanogen bromide (CNBr). Alternatively, polypeptide fragments can be generated in a highly acidic environment, for example at pH 2.5. Such

polypeptide fragments may be equally well prepared by chemical synthesis or using hosts transformed with an expression vector containing nucleic acids encoding polypeptide fragments according to the invention. The transformed host cells contain a nucleic acid and are cultured according to well-known methods; thus, the invention allows for the expression of these fragments, under the control of appropriate elements for regulation and/or expression of the polypeptide fragments.

[0078] Modified polypeptides according to the invention are understood to designate a polypeptide obtained by variation in the splicing of transcriptional products of the *lrba* gene, genetic recombination, or by chemical synthesis as described below. Modified polypeptides contain at least one modification in relation to the normal polypeptide sequence. These modifications can include the addition, substitution, deletion of amino acids contained within the polypeptides of the invention.

[0079] Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the polypeptide. For example, the class of nonpolar amino acids include Ala, Val, Leu, Ile, Pro, Met, Phe, and Trp; the class of uncharged polar amino acids includes Gly, Ser, Thr, Cys, Tyr, Asn, and Gln; the class of acidic amino acids includes Asp and Glu; and the class of basic amino acids includes Lys, Arg, and His. In some instances, non-conservative substitutions can be made where these substitutions do not significantly detract from the biological activity of the polypeptide.

[0080] In order to extend the life of the polypeptides of the invention, it may be advantageous to use non-natural amino acids, for example in the D form, or alternatively amino acid analogs, such as sulfur-containing forms of amino acids. Alternative means for increasing the life of polypeptides can also be used in the practice of the instant invention. For example, polypeptides of the invention, and fragments thereof, can be recombinantly modified to include elements that increase the plasma, or serum half-life of the polypeptides of the invention. These elements include, and are not limited to, antibody constant regions (see for example, U.S. Patent No. 5,565,335, hereby incorporated by reference in its entirety, including all references cited therein), or other elements such as those disclosed in U.S. Patent Nos. 6,319,691; 6,277,375; or 5,643,570, each of which is incorporated by reference in its entirety, including all references cited within each respective patent. Alternatively, the polynucleotides and genes of the instant invention can be recombinantly fused to elements

that are useful in preparation of immunogenic constructs for the purposes of vaccine formulation or elements useful for the isolation of the polypeptides of the invention.

[0081] The polypeptides, fragments, and immunogenic fragments of the invention may further contain linkers that facilitate the attachment of the fragments to a carrier molecule for the stimulation of an immune response or diagnostic purposes. The linkers can also be used to attach fragments according to the invention to solid support matrices for use in affinity purification protocols. In this aspect of the invention, the linkers specifically exclude, and are not to be considered anticipated, where the fragment is a subsequence of another peptide, polypeptide, or protein as identified in a search of protein sequence databases as indicated in the preceding paragraph. In other words, the non-identical portions of the other peptide, polypeptide, of protein is not considered to be a "linker" in this aspect of the invention. Non-limiting examples of "linkers" suitable for the practice of the invention include chemical linkers (such as those sold by Pierce, Rockford, IL), peptides that allow for the connection of the immunogenic fragment to a carrier molecule (see, for example, linkers disclosed in U.S. Patent Nos. 6,121,424; 5,843,464; 5,750,352; and 5,990,275, hereby incorporated by reference in their entirety). In various embodiments, the linkers can be up to 50 amino acids in length, up to 40 amino acids in length, up to 30 amino acids in length, up to 20 amino acids in length, up to 10 amino acids in length, or up to 5 amino acids in length.

[0082] In other specific embodiments, the polypeptides, peptides, derivatives, or analogs thereof may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (*e.g.*, a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art (see, for example, U.S. Patent No. 6,342,362, hereby incorporated by reference in its entirety; Altendorf *et al.* [1999-WWW, 2000] "Structure and Function of the F_o Complex of the ATP Synthase from *Escherichia Coli*," *J. of Experimental* Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] "Recombinant Protein Expression in *Escherichia coli*," *Biotechnology* 10:411-21, Elsevier Science Ltd.; Eihauer *et al.* [2001] "The FLAG™ Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins," *J. Biochem Biophys Methods* 49:455-65; Jones *et al.* [1995] *J. Chromatography* 707:3-22; Jones *et al.* [1995] "Current Trends in Molecular Recognition and Bioseparation," *J. Chromatography A.* 707:3-22, Elsevier Science B.V.; Margolin [2000]

"Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells," *Methods* 20:62-72, Academic Press; Puig *et al.* [2001] "The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification," *Methods* 24:218-29, Academic Press; Sassenfeld [1990] "Engineering Proteins for Purification," *TibTech* 8:88-93; Sheibani [1999] "Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins," *Prep. Biochem. & Biotechnol.* 29(1):77-90, Marcel Dekker, Inc.; Skerra *et al.* [1999] "Applications of a Peptide Ligand for Streptavidin: The *Strep*-tag", *Biomolecular Engineering* 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," *The* Scientist 12(22):20; Smyth *et al.* [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular Matrix Proteins Carrying the Strep II Tag", *Methods in Molecular Biology*, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and Purification Systems," *The Scientist* 11(17):20, each of which is hereby incorporated by reference in their entireties). Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. Fusion peptides can comprise polypeptides of the subject invention and one or more protein transduction domains, as described above. Such fusion peptides are particularly useful for delivering the cargo polypeptide through the cell membrane.

[0083] The expression of the *lrba* gene or *lrba* gene product (*e.g.*, DNA, RNA, or polypeptide) is disregulated in a variety of cancers, tumors, and/or malignancies. Non-limiting examples of such cancers, tumors, and/or malignancies include prostate cancer, breast cancer, melanoma, chronic myelogenous leukemia, cervical cancer, adenocarcinomas, lymphoblastic leukemia, colorectal cancer, and lung carcinoma. Accordingly, the present invention provides a method for screening, or aiding in the diagnosis of, an individual suspected of having a malignancy or cancer. The subject invention provides methods comprising the steps of determining the amount of *lrba* in a biological sample obtained from said individual and comparing the measured amount of *lrba* to the amount of *lrba* found in the normal population. The presence of a significantly increased amount of *lrba* is associated with an indication of a malignancy or cancer. *Lrba* gene product can be detected by well-known methodologies including, and not limited to, Western blots, enzyme linked immunoassays (ELISAs), radioimmunoassays (RIAs), Northern blots, Southern blots, PCR-based assays, or other assays for the quantification of gene product known to the skilled

artisan. This information, in conjunction with other information available to the skilled practitioner, assists in making a diagnosis.

[0084] Antisense technology can also be used to interfere with expression of the disclosed polynucleotides. For example, the transformation of a cell or organism with the reverse complement of a gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and silencing or inhibition of a target gene, *e.g.*, one involved in the infection process.

[0085] Polynucleotides disclosed herein are useful as target genes for the synthesis of antisense RNA or dsRNA useful for RNA-mediated gene interference. The ability to specifically inhibit gene function in a variety of organisms utilizing antisense RNA or ds RNA-mediated interference is well known in the fields of molecular biology (see for example C.P. Hunter, Current Biology [1999] 9:R440-442; Hamilton et al., [1999] Science, 286:950-952; and S.W. Ding, Current Opinions in Biotechnology [2000] 11:152-156, hereby incorporated by reference in their entireties). dsRNA (RNAi) typically comprises a polynucleotide sequence identical or homologous to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules and should not hybridize with sequences within the hybridizing portions of the dsRNA molecule. The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. Accordingly, one method for controlling gene expression according to the subject invention provides materials and methods using double-stranded interfering RNA (dsRNAi), or RNA-mediated interference (RNAi). The terms "dsRNAi", "RNAi", "iRNA", and "siRNA" are used interchangeably herein unless otherwise noted.

[0086] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and

references cited t[redacted]n) and calculating the percent differe[redacted] between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (*e.g.*, University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[0087] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (*e.g.*, promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands); the promoters may be known inducible promoters such as baculovirus. Inhibition may be targeted by specific transcription in an organ, tissue, or cell type. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (*e.g.*, T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no, or a minimum of, purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[0088] Preferably and most conveniently, dsRNAi can be targeted to an entire polynucleotide sequence set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides of the sequence listing. The homology may be greater than 70%, preferably greater than 80%, more preferably greater than 90% and is most preferably greater than 95%.

[0089] Fragments of genes can also be utilized for targeted suppression of gene expression. These fragments are typically in the approximate size range of about 20

nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. In a more preferred embodiment, the gene fragments are at least about 25 nucleotides in length. In an even more preferred embodiment, the gene fragments are at least 50 nucleotides in length.

[0090] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. Gene product can be inhibited with an RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or greater identity is also preferred, but not necessarily essential, for such applications.

[0091] In another aspect of the invention, the dsRNA molecules of the invention may be introduced into cells with single stranded (ss) RNA molecules which are sense or anti-sense RNA derived from the nucleotide sequences disclosed herein. Methods of introducing ssRNA and dsRNA molecules into cells are well-known to the skilled artisan and includes transcription of plasmids, vectors, or genetic constructs encoding the ssRNA or dsRNA molecules according to this aspect of the invention; electroporation, biolistics, or other well-known methods of introducing nucleic acids into cells may also be used to introduce the ssRNA and dsRNA molecules of this invention into cells.

[0092] As used herein, the term "administration" or "administering" refers to the process of delivering an agent to a patient, wherein the agent directly or indirectly suppresses lrba function and inhibits the growth of tumors. The process of administration can be varied, depending on the agent, or agents, and the desired effect. Administration can be accomplished by any means appropriate for the therapeutic agent, for example, by parenteral, mucosal, pulmonary, topical, catheter-based, or oral means of delivery. Parenteral delivery can include for example, subcutaneous intravenous, intrauscular, intra-arterial, and injection into the tissue of an organ, particularly tumor tissue. Mucosal delivery can include, for example, intranasal delivery. Oral or intranasal delivery can include the administration of a propellant. Pulmonary delivery can include inhalation of the agent. Catheter-based delivery can include delivery by iontropheretic catheter-based delivery. Oral delivery can include delivery of a coated pill, or administration of a liquid by mouth. Administration can generally also include delivery with a pharmaceutically acceptable carrier, such as, for example, a buffer, a polypeptide, a peptide, a polysaccharide conjugate, a liposome, and/or a lipid. Gene therapy protocol is also considered an administration in which the therapeutic

agent is a polynucleotide capable of accomplishing a therapeutic goal when expressed as a transcript or a polypeptide into the patient.

[0093] As used herein, the term "biological activity" with respect to the nucleotides and polypeptides of the subject invention refers to the inhibition of tumor cell growth or proliferation. Thus, cell-based assays can be utilized to determine whether an agent, such as nucleotide or polypeptide, can be utilized to carry out the method of tumor growth inhibition of the subject invention, as shown in Figures 18A-21D.

[0094] The term "means for inhibiting or suppressing *lrba* function" comprises genetic and non-genetic means for inhibiting or suppressing *lrba* function. Among the genetic constructs inhibiting *lrba* function are various "gene delivery vehicles" known to those of ordinary skill in the art, that facilitate delivery to a cell of, for example, a coding sequence for expression of a polypeptide, such as an *lrba* inhibitor, an anti-sense oligonucleotide, an RNA aptamer capable of inhibiting *lrba* function, or other genetic construct capable of inhibiting lrba function at the transciption, translation, or post-translation level. Methods of gene silencing and/or knock-down, as described herein, and as known to those of ordinary skill in the art, can be utilized to suppress *lrba* function, for example. For example, gene therapy comprising administration of a dominant negative *lrba* mutant can be utilized to carry out the method of tumor inhibition of the subject invention.

[0095] Among the non-genetic means for inhibiting *lrba* function are pharmaceutical agents, or pharmaceutically acceptable salts thereof, which are preferably administered in a pharmaceutically acceptable carrier.

[0096] The term "patient", as used herein, refers to any vertebrate species. Preferably, the patient is of a mammalian species. Mammalian species which benefit from the disclosed methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales.

[0097] The terms "lrba", "LRBA", and "Lrba" (italicized and unitalicized) are used herein interchangeably to refer to the LPS-responsive CHS1/beige-like gene or its

polypeptide produ[redacted]nd includes *lrba* homologs (such as h[redacted]n and mouse orthologs), unless otherwise noted.

[0098] The terms "comprising", "consisting of", and "consisting essentially of" are defined according to their standard meaning and may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

Materials and Methods

[0099] Murine RNA Isolation and cDNA Synthesis. Total RNA was prepared using the RNEASY kit (QIAGEN, Valencia, CA). Poly(A)$^+$ RNA was prepared using the FAST TRACK mRNA isolation kit (INVITROGEN, Calsbad, CA). RNA was prepared from murine cell lines as well as liver and thymus of C57BL6/J mice per the manufacturers' instructions. RNAs were treated with Rnase-free Dnase I (AMERSHAM PHARMACIA BIOTECH, Piscataway, NJ) at 10 U/μg of RNA for 30 minutes at 37° C to destroy genomic DNA. First-strand cDNA synthesis was primed with random DNA hexamers or oligo(dT) primers at 42° C for 1 hour using the SUPERSCRIPT II RNase H Reverse Transcriptase cDNA Synthesis System (Life Technologies, Inc., Rockville, MD).

[00100] Cloning and Sequencing of Murine *lrba* Gene cDNAs. Primers (5'AGAGAAGAGGAGAAGATGTGTGATC3'; and 5'CCAGGCTCCATGCTTGTCTGTGAG3' forward and reverse, respectively) were designed from a 143 bp cDNA fragment obtained from previous gene-trap experiments (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947) and combined with Lambda GT10 forward and reverse primers (5'AGCAAGTTCAGCCTGGTTAAGT3' and 5'TTATGAGTATTTCTTCCAGGG3', respectively) to amplify the *lrba* gene cDNA from a mouse B lymphocyte cDNA library (Mouse lymphocyte 5' stretch cDNA library, CLONTECH, Palo Alto, CA). These PCR products were then cloned and sequenced. New primers were then designed from these sequences and further RT-PCR reactions were carried out to extend the cDNA sequence to the 5' or 3' direction. The SMART RACE amplification kit (CLONTECH, Palo Alto, CA) was used to amplify 5' cDNA ends using the following *lrba*-specific primers: 5'ACTGCAGCAAGCTCCTCCTGTTTTCTC3' and a nested primer: 5'TGGGCGAAGAGCGGAAACAGAAC3', while for 3' cDNA clones the following primers were used: 5'AGAGAAGAGGAGAAGATGTGTGATC3' and a nested primer: 5'GAGTGATGGATGATGGGACAGTGGTG3'. PCR conditions for the 5'-RACE and 3'-

RACE were as follows using the ADVANTAGE polymerase mix (CLONTECH, Palo Alto, CA): 94° C for 30 seconds, followed by 5 cycles at 94° C for 30 seconds, 70° C for 30 seconds, and 72° C for 3-5 minutes; 5 cycles at 94° C for 30 seconds, 68° C for 30 seconds, and 72° C for 3-5 minutes; 20 cycles at 94° C for 30 seconds, 65° C for 30 seconds, and 72°C for 3-5 minutes; and a final extension at 72° C for 30 minutes. After the full-length cDNA sequence of the *lrba* gene was obtained, several primers were designed to amplify the region of the *lrba* gene cDNA containing its major open reading frame (ORF). The region containing the major ORF of the *lrba* gene was then amplified from a single source of C57BL6/J liver mRNA and resequenced to confirm that the *lrba* cDNAs obtained from liver cells were identical to that amplified from the aligned cDNA fragments amplified from primary and transformed B lymphocytes, indicating that these represent the major mRNAs expressed from the *lrba* locus. All RT-PCR and RACE products were isolated and purified from agarose gels using the QIAEX II Gel Extraction Kit (QIAGEN; Valencia, CA). The purified products were sequenced directly to avoid detecting the mutations introduced during PCT. Both strands of each template were sequenced and the sequence was confirmed by sequence analysis of at least two independent PCR products. PCR products and RACE products were cloned into PCRII vector (TA cloning kit; INVITROGEN, Carlsbad, CA) and multiple clones were sequenced. Plasmids were purified from liquid cultures using the QIAGEN plasmid Maxi preparation kit (QIAGEN; Valencia, CA).

[00101] Human *lrba* cDNA Cloning and Sequencing. A search of GENBANK indicated that the murine *lrba* gene has a high degree of homology to a 7.3 kb human partial cDNA sequence (GENBANK accession numbers M83822) called BGL (Feuchter, A.E. *et al. Genomics*, 1992, 13:1237), which was thereby tentatively identified as possibly a small fragment of a human *lrba* gene. The 5' end of the human *lrba* gene was obtained by using a 5' primer (5'GCCACCTCCGTCTCGCTGC3') from the mouse *lrba* gene cDNA sequence and a 3' primer (5'GGGCACTGGGGAGAATTTCGAAGTAGG3') from the human BGL sequence. Human lung, brain, and kidney cDNA libraries (MARATHON cDNA Libraries, CLONTECH, Palo Alto, CA) were used as templates for the amplification of the 5' and 3' ends of the human cDNA under the following PCR conditions: 35 cycles at 95° C for 45 seconds; 60° C for 15 seconds; 72° C for 3 minutes. The PCR products were cloned into a TA cloning vector and multiple clones were sequenced. Additional PCRs were carried out with the primers from the 3' cDNA clones obtained as described above to complete the sequence of the human *lrba* cDNA. The primer pairs used for these additional 3' cDNA

clones w[redacted] 5'TTCAGGCAGTTTTCAGGA[redacted]TCCAAG3' and 5'TAGTGTCTGATGTTGAACTTCCTCCTG3'. Overlapping regions of the 5' and 3' human *lrba* cDNAs were compared and merged with the human BGL cDNA in GENBANK to construct, for the first time, a complete sequence for the human *lrba* gene (GenBank accession number AF216648). The human *lrba* gene encodes a 319KD protein that has 2863 amino acids. The amino acid homology between the human and murine *lrba* gene is 93% (identity 89%, similarity 4%). Like the murine *lrba* gene, the human *lrba* gene contains BEACH domain, five WD40 repeats and two novel domains that are defined as followed (Figures 9 and 10).

[00102] Northern Blot Analysis. 70Z/3 B lymphoma cells were maintained in RPMI1640 supplemented with 10^{-5}M 2-mercaptoethanol and 10% fetal bovine serum (FBS). J774 cells were maintained in DMEM supplemented with 10% FBS. 70Z/3 cells were stimulated with 10 ng/ml LPS (Sigma, St. Louis, MO) and J774 cells were stimulated with 1 ng/ml LPS for 20 hours. Poly(A)$^+$ RNA was prepared from 10^8 stimulated or unstimulated cells using the FASTRACK isolation kit (INVITROGEN, Carlsbad, CA). Poly(A)$^+$RNA (5 μg/lane) was size-fractionated by electrophoresis on a 6% formaldehyde/ 1% agarose gel buffered with MOPS, transferred to a nylon membrane (STRATAGENE, La Jolla, CA) by capillary action in 20X SSC and immobilized by UV cross-linking. The filter was probed with a uniformly labeled ^{32}P probe using the READY-TO-GO DNA labeling kit (AMERSHAM PHARMACIA BIOTECH, Piscataway, NJ). The probe corresponds to a 2.5 kb PCR product that spans nucleotides 3545-6040 of the murine *lrba* cDNA. The filter was hybridized with the probe in 2XSSC, 0.5% SDS, 5X Denhardt's containing 100 μg/ml heat denatured salmon sperm DNA at 68° C overnight. Filters were washed 2 times for 5 minutes at room temperature in 2X SSC/0.5% SDS and 2 times for 30 minutes at 68° C in 0.1X SSC/0.1% SDS. Hybridization signals were detected and quantitated using a Molecular Dynamics PHOSPHORIMAGER and IMAGEQUANT software.

[00103] RT-PCR Analysis of *lrba* Expression. The cell lines (70Z/3, BAL17, A20, WEHI231, and S194) used for the RT-PCR were obtained from ATCC (Rockville, MD). Spleen, brain, lung, and bone marrow were obtained from C57BL6/J mice. The preparation of total RNA and cDNA synthesis were carried out as described above. First strand cDNA reaction products (2 μl) were amplified in a 25 μl PCR reaction using primers that detect three of the *lrba* isoforms ("5'GGCACAACCTTCCTGCTCAC3'" and "5'CCTGTCCCCCATTTGAACCC3'" for the α form: "5'ACGGCTGCTTCTGCACCTTC3'"

and "5'TTT[redacted]GACAGGGCTTCTCTG3'" for [redacted] the β form; "5'GGCACAACCTTCCTGCTCAC3'" and "5'GCAGATGCTCTCCTCGCTCC3'" for the γ form). The cycling program was: 94° C for 30 seconds, followed by 5 cycles at 94° C for 30 seconds, 70° C for 30 seconds, and 72° C for 4 minutes; 5 cycles at 94° C for 30 seconds, 68° C for 30 seconds, and 72° C for 4 minutes; 30 cycles at 94° C for 30 seconds, 62° C for 30 seconds, and 72° C for 4 minutes; and a final extension at 72° C for 10 minutes.

[00104] Gene and Protein Structure Prediction. Analyses of the nucleotide and amino acid sequences for the murine and human *lrba* gene were performed using MACVECTOR (Oxford Molecular Group Inc., Oxford, UK). Nucleotide sequence alignments and other analyses were carried out using BLAST (Altschul, S.F. and E.V. Koonin *Trends in Biochemical Sciences*, 1998, 23:444). SMART (Schultz, J. *et al. Nucleic Acids Res.*, 2000, 28:231), and CLUSTLX (Thompson, J.D. *et al. Clinical Orthopaedics & Related Res.*, 1997, 241) were used for protein secondary structure predictions. For WD repeat prediction, an algorithm developed by Neer *et al* (Neer, E.J. and T.F. Smith *Cell*, 1996, 84:175; Garcia-Higuera, I. *et al. Biochemistry*, 1996, 35:13985; Neer, E.J. *et al. Nature*, Oct. 1994, 371(6500):812; Smith, T.F. *et al. Trends Biochem.*, 1999, 24:181; Neer, E.J. and T.F. Smith *Proc. Natl. Acad. Sci. USA*, 2000, 97:960) is used.

[00105] Construction, Expression, and Fluorescence Microscopy of the *Lrba*-GFP Fusion Protein. A region from the murine *lrba* cDNA that includes the BEACH and the WD domains 3' to the BEACH domain was inserted "in-frame" and upstream of the coding region of a modified GFP gene cloned in a mammalian expression vector pEGFP-N2 (CLONTECH, Palo Alto, CA). Recombinant clones (called pBWEGFP) were picked, plasmid DNAs prepared and sequenced to confirm that no mutations were introduced during these manipulations. Murine 3T3 cells, the macrophage RAW264.7 cells, and human 293 cells were transfected by the FUGEN transfection kit (ROCHE Molecular Biochemicals, Indianapolis, IN) or by electroporation (Gene Pulser; BIO-RAD Laboratories, Hercules, CA) with 20 μg of linearized recombinant plasmid pBWEGFP DNA as well as the control vector pEGFP at 250V, 500μF. One day later, cells were cultured in DMEM containing 0.8 μg/ml of G418 (LIFE TECHNOLOGIES, Inc., Rockville, MD). This medium was changed every day for the first four days. The surviving G418 resistant colonies were isolated and used for further experimentation. For subcellular localization, cells were plated in glass-covered plates at 2.5 x 10^5 cells/ml in 2 ml DMEM media with or without LPS at 100 ng/ml. After 12 hours, cells were directly examined by fluorescence microscopy using a fluorescein

isothiocyanate fi[redacted]to detect expression of GFP fus[redacted]proteins. Fluorescent photomicrography was performed using Nikon photomicrographic equipment model H-III and image software (NIKON, Tokyo, Japan).

[00106] Confocal Laser Scanning Microscopy. The RAW 264.7 cells stably transfected with the pBWEGFP construct were grown on glass coverslips and stimulated with 100 ng/ml LPS for 24 hours. Golgi and lysosomes were specifically labeled with BODIPY TR ceramide and LysoTracker Red DND-99 (MOLECULAR PROBE, Eugene, OR), respectively, following the manufacturer's protocols. Briefly, for Golgi labeling, cells were washed with PBS three times and incubated for 30 minutes at 4° C with 5 μM BODIPY TR ceramide, rinsed several times with ice-cold medium, and then incubated in fresh medium at 37° C for another 30 minutes. For lysosome labeling, medium was changed with pre-warmed fresh medium containing 60-75 nM lysosome probe and the cell sample was incubated for 30 minutes. Finally, the medium was removed, washed with PBS three times, fixed with 3.7% formaldehyde for 10-20 minutes, washed again, and the slides were mounted with DAPI-containing VECTASHIELD medium (VECTOR LABORATORIES, Burlingame, CA). Cells were observed on a Zeiss inverted Axiovert 100 M laser scanning confocal microscope. Fluorescence of GFP was excited using a 458/488 nm argon/krypton laser, and emitted fluorescence was detected with 505-530 nm band pass filter. For LysoTracker Red and BODIPY TR, a 633-nm helium/neon laser was used for excitation, and fluorescence was detected with a 585 nm band pass filter, using a 100X oil immersion lens. The co-localization function of LSM510 software (EMBO Laboratory) allows for a reliability of 99% for actual pixels with both fluorophores. The co-localization mask pixels were converted to white color for clarity.

[00107] Immunoelectron Microscopy. The RAW 264.7 cells stably transfected with the pBWEGFP construct were grown in the presence of 100 ng/ml LPS for 24 hours, washed with PBS three times, fixed with 2% paraformaldehyde in phosphate buffer for 1 hour and 4° C, and processed for postembedding immunocytochemistry. The cells were scraped from the dishes they were grown in and pelleted by low speed centrifugation. The pellets were dehydrated in a graded series of ethanol dilutions and embedded in gelatin capsules in LR White resin. The resin was polymerized for 48 hours at 50° C. Ultrathin sections of LR White embedded cells were collected on nickel grids and immunolabeled according to the technique of Haller *et al.* (Haller, E.M. *et al. J. Histochem Cytochem*, 1992, 40:1491) with rabbit-anti-GFP (CLONTECH, Palo Alto, CA) at 1:20 ration for 1 hour at room temperature,

followed by exte[redacted] rinsing and then labeling with 10 n[redacted]oat-anti-rabbit IgG-gold (AURION, Wageningen, The Netherlands) for 1 hour at room temperature. Control grids were labeled by replacing the primary antibody with normal rabbit serum. After extensive washing, thin sections were stained with uranyl acetate and lead citrate before examination with EM.

[00108] Primers. The gene-specific primers were designed from the partial sequences of the human *lrba* that were obtained and from BGL sequence in the GenBank (GenBank accession numbers M83822). The sequences of synthetic oligonucleotides used for PCR amplification were as follows: cdc415mar2: CACACAGAGCATTGTAGCAAGCTCCTC; h65-56153: TGCAGACTTGAAGATTCCG; 3CDS: AAGCAGTGGTATCAACGCAGAGTACGCAGAGTACTVN; h6439: GAGTGATGGATGATGGGACAGTAGTG; cdc415mar1: GGGCACTGGGGAGAATTTCGAAGTAGG; and h5end65': CGAGAAGATGAGAAGATGTGTGATC.

[00109] Human RNA isolation and cDNA synthesis. Total RNA was prepared using the RNeasy kit (QIAGEN, Valencia, CA). RNA was prepared from cell lines as well as human prostate tumor tissues and normal adjacent tissue per the manufacturers' instructions. First-strand cDNA synthesis was primed with gene-specific primers or oligo(dT) primers at 42°C for 1h-2h using the SUPERSCRIPT II RNase H Reverse Transcriptase cDNA Synthesis System (Life TECHNOLOGIES, Inc., Rockville, MD) or PowerScript Reverse Transcriptase (CLONTECH, Palo Alto, CA).

[00110] 5'-RACE, 3'-RACE and the Cloning of human *lrba* Gene cDNAs. 5'-RACE, 3'-RACE of h*lrba* gene were carried out by using the SMART RACE amplification kit (CLONTECH, Palo Alto, CA) and the following condition: 5'-RACE : cdc415mar2 as reverse transcription primer, 1- 2.5 μg RNAs were used. cdc415mar1 was used for first PCR reaction, h65-56153 () was used for nested primer; 3'-RACE: 3CDS from the kit was used as reverse transcription primer. h5end65' was used for first PCR reaction and h6439 was used for nested PCR primer. The PCR parameters are: 94 °C for 30 seconds, followed by 5 cycles at 94°C for 30s, 70°C for 30s, and 72°C for 3-5 min; 5 cycles at 94°C for 30s, 68°C for 30s, and 72°C for 3-5 min; 25 cycles at 94°C for 30s, 65°C for 30s, and 72°C for 3-5 min; and a final extension at 72°C for 10 min. All RT-PCR and RACE products were isolated and purified from agarose gels using the QIAEX II Gel Extraction Kit (QIAGEN; Valencia, CA). The purified products were sequenced directly to avoid detecting the mutations introduced during

PCR. Both strands of each template were sequenced and the sequence was confirmed by sequence analysis of at least two independent PCR products. PCR products and RACE products were cloned into PCRII vector (TA cloning kit; INVITROGEN, Carlsbad, CA) and multiple clones were sequenced.

[00111] Mapping of the 5' end of the human *lrba* gene. The 5' end of the human *lrba* gene were determined by SMART 5' RACE (Clontech, Palo Alto, CA) in tumor tissues and adjacent tissues from prostate, human lung carcinoma, B-cell lymphoma and B-cell lymphoma (AMBION, Austin, TX). cdc415mar1 as reverse transcription primer were used. The *lrba* gene-specific primer cdc415mar2 was used to prime reverse transcription using 1-2.5 μg RNAs. Then first PCR reaction was performed using gene-specific primer cdc415mar2, h65-56153 was used for nested primer. Products were sequenced both directly and indirectly by first cloning into pCR2.1 vector (TA cloning kit; INVITROGEN, Carlsbad, CA).

[00112] Multiple Sequence Alignment. All amino acid sequences were obtained from the SWISS-PROT/TrEMBL database at the Expasy web site (www.expasy.ch). Homologous sequences were searched for using the BLAST server of Expasy. To gather tetraspanin and tetraspanin-like sequences from the data base, BLAST searches were performed using a number of sequences from well established members of the tetraspanin superfamily (*i.e.* CD81, CD82, CD9, CD53, CD63, UPK, RDS, and ROM). A multiple sequence alignment was initially achieved with the CLUSTALIX software. The alignment was then improved manually using the GENEDOC software.

[00113] Secondary Structure Prediction. To predict the secondary structure of the HSH domain, two methods (available on the World Wide Web) based on a consensus assignment were used. The first method, Jpred[2], takes a multiple sequence alignment as input and performs a consensus average of nine different alignment-based secondary structure prediction methods. Alignment-based prediction methods have been demonstrated to have a significantly better accuracy than those using single sequences, and consensus averaging by Jpred[2] has been shown to increase the accuracy to 72.9%. The use of alignment-based secondary structure prediction methods requires the sequences to have a degree of homology of at least ~25%.

[00114] RT- PCR Analysis of *hlrba* Expression. The cell lines MCF7 breast cancer cell line, 293 cell line, pre-B (6417); Raji B cells; HTB4 lung cancer; H322 human lung cancer; A539 human lung cancer used for the RT-PCR were obtained from ATCC

(Rockville, MD). [redacted] preparation of total RNA and cDNA [redacted]esis were carried out as described above. First strand cDNA reaction products (2μl) were amplified in a 25μl PCR reaction using primers.

[00115] Following examples illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1--Cloning and Sequencing of the Murine *lrba* cDNA

[00116] An LPS-inducible gene was identified by integration of Gensr1 gene-trap retrovirus (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947). A partial cDNA sequence of the LPS-inducible gene-trap cell clone, 7a65, was used to design PCR primers to amplify the upstream and downstream regions of cDNA from a mouse B lymphocyte library. Initially, a 1.6 Kb cDNA sequence was obtained by this strategy. Sequence analysis confirmed that this 1.6 Kb cDNA sequence contains the original 142 bp sequence obtained by gene-trapping (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947). 5' RACE reactions using anti-sense primers from the 5' end of this 1.6 Kb region yield additional 5' cDNA sequences including the 5' UTS of the *lrba* gene as well as the ATG of its major ORF. Sense strand primers were also designed from the 1.6 Kb cDNA sequence and three 3' RACE fragments of 2.5 Kb, 2 Kb, and 1.4 Kb were obtained that have identical 5' end sequence; however, their 3' ends differ substantially. The amino acid sequence of the major ORF in the murine *lrba* cDNA is shown in Figure 1A. The human *lrba* orthologue is obtained as described in the Experimental Procedures section.

[00117] Sequence analysis of the *lrba* cDNAs indicated the existence of three isoforms with identical 5' ends that differ at their 3' termini. These isoforms include a 9903 bp form (*lrba-α*), a 9396 bp form (*lrba-β*) and 8854 bp form (*lrba-γ*) encoding proteins of 2856, 2792, and 2779aa, respectively. All three ORFs begin with the same Kozak consensus ATG at nucleotide 308. The first 2776aa of the β form are identical to the first 2776aa of the α form, while the 16aa at its C-terminus are unique to it. The first 2769aa of the γ form are identical to the first 2769aa of the α and β forms with its C-terminal 10aa unique to it; the α form has its C-terminal 80aa unique to it (Figure 1). Homology search indicates that all *lrba* isoforms have a BEACH domain (Nagle, D.L. *et al. Nature Genetics*, 1996, 14:307); however, the *lrba-α* isoform has 5 WD repeats, *lrba-β* has 3 WD repeats while *lrba-γ* lacks WD repeats (Figure 1B). The isoform specific unique coding sequences and the associated 3'

untranslated sequences totally 1267 bp for α form, 761 bp for β form, and 845 bp for γ form) show no significant homology with each other. Interestingly, only the α form has an AATAAA sequence for polyA recognition and a TGA stop codon, while the β and γ forms have TAA stop codons.

Example 2--*Lrba* Orthologues Exist in Diverse Organisms and Belong to a Novel Gene Family

[00118] Homology analysis revealed that *lrba* has significant homology with the partial protein sequence DAKAP550 (Han, J.D. *et al. Jour. Biol. Chem.*, 1997, 272:26611), which is an AKAP, and with AKAP550 (GENBANK accession number AAF46011) predicted from the *Drosophila* genomic sequence (GENBANK accession number AE003433). A longer sequence for this gene is predicted from the genomic sequence and is designated dLRBA, which is identical to AKAP550 except that it has an additional 160aa at its N-terminus. As used herein, the first letter of the genus is placed before the gene's name to distinguish the *lrba* genes of different species. Thus, DAKAP550 is a partial sequence of dLRBA and AKAP550. Amino acid alignment analysis shows that the murine LRBA protein has 85% aa identity with human LRBA, 51% aa identity with dLRBA and 35% aa identity with the *C. elegans* CDC4L gene (GENBANK accession number T20719) (designated cLRBA for clarity) (Figure 1B). This homology analysis shows that the *lrba* and DAKAP550 genes are othologues based on their high homology that extends from their N terminus to the C terminus (Figures 1-3 and Table 1). Furthermore, two putative PKA binding sites are found in all *lrba* orthologues (Figures 2A and 2B) and are structurally similar to the B1 and B2 RII binding sites of DAKAP550, a protein that has been demonstrated to bind PKA *in vitro* and *in vivo* (Han, J.D. *et al. Jour. Biol. Chem.*, 1997, 272:26611). This region is highly conserved in *lrba* orthologues in mice, man, *Drosophila*, and *C. elegans* (Figure 2A) and potentially provides another two PKA binding sites for DAKAP550. Unexpectedly, the B1 and B2 sites of DAKAP550 are not found in other LRBA proteins; they may be species-specific and these potential RII binding sites need to be confirmed by biochemical studies.

[00119] Table 1 shows the protein homology between LRBA and dLRBA, mBG, and hFAN, showing the precentage of identity, and positive gaps. The positions of each fragment are also indicated.

Table 1				
mLBA dLBA	Identities	Positives		Length (aa)
92-405	47-394	51%	73%	314
405-959	601-1160	55%	75%	555
998-1576	1542-2127	36%	53%	579
1793-2856	2642-3727	56%	74%	1064
mLBA cLBA				
65-946	164-1057	42%	61%	882
1300-1571	1065-1333	39%	59%	271
1787-2856	1436-22512	47%	64%	1070
mLBA hLBA				
1-2856	1-2863	85%	88%	2856
mLBA mBG				
1934-2839	1460-2335	27%	43%	906
mLBA hFAN				
2038-2841	163-913	29%	45%	803

[00120] These *lrba* orthologues also have a highly conserved long C-terminal region (around 1000 amino acids) shared with a group of proteins including CHS1/BG (Perou, C.M. *et al. Nature Genetics*, 1996, 13:303; Kingsmore, S.F. *et al. Jour. Invest. Med.*, 1996, 44:454), FAN (Adam-Klages, S. *et al. Cell*, 1996, 86:937), LVSA (Kwak, E. *et al. Cell*, 1999, 10:4429) proteins (Figures 2A and 2B), and a number of anonymous ORFs. They constitute a new gene family. The conserved region contains an unidentified region followed by one BEACH domain and several WD repeats. Several WD repeats are found in the unidentified region of homology in these genes when about 1000 aa of C-terminal sequence is searched for WD repeats; however, no WD repeat is predicted when this region is analyzed alone (data not shown). Thus, this region is designated herein as WD repeat-like domain (WDL). In aggregate, and not to be limited by theory, the entire WDL-BEACH-WD (WBW) structure may have a precise functional role since the WD repeats found in the WBW structures of different *beige*-like genes have a higher degree of homology with each other than with other WD repeats in proteins that lack a BEACH domain (Figure 3). This homology analysis suggests the evolutionary conservation of the WBW structure in a gene family that includes *lrba*, *chs1/beige*, FAN, *lvsA*, and other unidentified ORFs in GENBANK. However, the BEACH domain can exist without WD motifs as in the case of *lrba-γ* (Figures 1A, 1B and 3). It is shown herein that all BEACH domains have an SH3 binding site (consensus sequence PXXP), an SH2 binding site (consensus sequence YXXhy) (Pawson, T. and J.D. Scott *Science*, 1997, 278:2075), and a tyrosine kinase phosphorylation site (consensus sequence: (RK)-x(2,3)-(DE)-x(2,3)-Y) (Patschinsky, T. *et al. Proc. Natl.*

Acad. Sci. USA, 19[redacted]9:973; Hunter, T. *J. Biol. Chem.*, 1982,[redacted]4843; Cooper, J.A. *et al. J. Biol. Chem.*, 1984, 259:7835), as shown in Figure 3. These putative binding sites show that WBW proteins may interact with multiple signal transduction components.

Example 3--Analysis of *lrba* mRNA Expression

[00121] Northern blot analysis indicates a single mRNA of about 10 Kb encoding the *lrba* gene is present in LPS-induced J774 macrophages and 70Z/3 B cells (Figure 4A), as well as in other B cell lines (WEHI231, BCL1) and the macrophage cell line, RAW264.7 [RAW267.4]. The size (~10 Kb) of the transcript is consistent with the cDNA sequence analysis described herein (9903 bp for *lrba-α*). The expression of the *lrba* gene is significantly up-regulated in LPS-induced J774 macrophage cells as the *lrba* mRNA is nearly undetectable in J774 cells in the absence of LPS stimulation. The level of *lrba* mRNA is increased by 3 fold in 70Z/3 B cells (Figure 4A) using β-actin mRNA as an internal standard. The upregulation of *lrba* expression in the B cell lines is consistent with the FACS analysis of *lacZ* induction in the 7a65 gene-trap cell clone (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947).

[00122] A multiplex RT-PCR assay was also developed that can simultaneously detect the expression of the *lrba* mRNA isoforms. RT-PCR analysis of *lrba* mRNA (Figures 4B and 4C) shows that *lrba-β* mRNA is expressed in all cell lines and tissues analyzed; however, *lrba-α* mRNA is absent in 70Z/3, lung and bone marrow and is less abundant in spleen and lung, suggesting that these different isoforms may have discrete functions in different tissues.

Example 4--Subcellular Localization of LRBA-GFP Fusion Protein Shifts Upon LPS Stimulation

[00123] All mutations in *beige* or chs1 genes result in truncated proteins that lack the BEACH and COOH terminal WD repeats (Certain, S. *et al. Blood*, 2000, 95:979). This region may contain sequences critical to the function of *chs1/beige* and *lrba* genes. In particular, the ability of their gene products to associate with intracellular vesicles to influence their trafficking may be lost in these truncated mutants. Therefore, a GFP fusion with the BEACH-WD region of *lrba* called BW-GFP was created. As shown in Figures 5A-5I, fluorescence microscopy of RAW 267.4 cells stably transfected with an expression vector encoding the BW-GFP fusion shows that the BW-GFP protein is present in the cytosol with

rare cells showing [redacted]sicular staining pattern in the absence of [redacted] stimulation (Figure 5A). However, this vesicular staining pattern is dramatically increased in these cells following LPS stimulation (Figure 5B). Both the percentage of cells and the degree of vesicular staining in each cell are increased following LPS stimulation. RAW267.4 cells stably transfected with a GFP control construct show no change in their GFP fluorescence pattern upon LPS stimulation (Figure 5C).

[00124] To determine which vesicular compartments the BW-GFP fusion localizes to, the RAW264.7 cells stably transfected with the pBWEGFP construct stained with a lysosome specific dye (Figure 5E) and trans-Golgi specific dye (Figure 5H) were analyzed with confocal microscopy. The merged pictures show that some LRBA-GFP proteins are co-localized with lysosomes (Figure 5F, white area) and co-localization with the trans-Golgi complex (Figure 5I, white peri-nucleus area).

[00125] Immunogold labeling experiments were also performed that show the LRBA-GFP fusion protein can be found in association with the Golgi complex (Figure 6D), lysosomes (Figure 6B and 6F), endoplasmic reticulum (Figure 6C), plasma membrane (Figure 6E), perinuclear ER (Figure 6E), and endocytic vacuole (Figure 6A, as the gold particles are labeling a clathrin coated endocytic vacuole, which indicates that it is involved in endocytosis and not exocytosis). The immunoelectron microscopy results agree well with the observations made by fluorescence microscopy and confocal fluorescence microscopy.

Example 5--Exon/Intron Structure of the human *lrba* gene

[00126] The genomic locus of *lrba* gene is composed of 58 exons and 57 introns, spinning over a 700 K bps genomic sequence. Exon 1 and exon 2 contain the first part of the 5' UTR, exon 2 contains the rest of the 5'UTR and the start methione, while exon 58, the final exon, contains part of the WD5 and the whole 3'UTR. There are two considerably large exons-- exon 24 (1059 bps) and exon 58 (1148 bps). The entire SET domain is encoded by one exon--exon 24, while other domains are econded by multiple exons. The remaining exons range in size from 33 to 435 bps, most are below 200 bps. All exon/intron junctions conform to the GT-donor/AG-acceptor rule (Breathnach and Chambon, 1981)(Table 1). The function of the *lrba* gene is defined by its domain structure consisting of BEACH domain, WD repeats, HSH domain and SET domain and potential RII binding sites. The BEACH domain is encoded by exons 45 to 51. The 5-WD repeat domain is encoded by exons 54 to 58. Isoforms are formed by splicing with splicing site inside the exons of the other isoforms.

[00127] Table 2 shows the exon/intron organization of the human *lrba* gene.

Table 2 - Exon/Intron Organization of the Human *lrba* Gene

Exon No	Exon size(bp)	5'Splice donor	Intron No	Intron size(kb)	3'Splice acceptor
1	~67	AGT ATC TGG gtgaggaag	I	0.340	tccaataag GGT TTG GCG
2	435	TTT AAC CTG gtaagtcca	II	85.572	ccttgtaag TTG GTA GGA
3	232	TGA TAG CAG gtatgattt	III	0.217	tgtttccag ATC TTT TGG
4	101	GGA CGA TGG gtaaaaaaa	IV	7.224	tcttcatag CCT CCA CAT
5	96	AGT GCT GCA gtaagtaa	V	4.458	ttcctttag GCT ATT GCA
6	122	TTT GTA TTG gtatgtatt	VI	0.089	tctttatag TTT CAG AAC
7	127	CCA CAA AAG gtacatgat	VII	0.674	cttctgcag TGG TAT ATG
8	120	ACT AGC GAT gtaagtagt	VIII	1.266	cttttacag ACC TTT GAC
9	147	GGA TAC AAG gtagtttgc	IX	5.537	ttcttagag GGT ACA TTT
10	198	ATG CTC CAG gtactaact	X	0.192	tcttacaag GAT GTA AAG
11	134	GAC TAT ATG gtgagtgcc	XI	1.971	aaattctag TTC AAC CTT
12	109	CTT GAA AAG gtaaagtat	XII	0.306	tttttgcag TCT TCC AAA
13	153	CCA GCC AAG gtaatatat	XIII	5.619	attctgtag GTT CAA CTG
14	169	AAG GAT TAG gtatataat	XIV	2.233	ttttaaaag ATG GAC CGC
15	80	GTG ATG AAG gtaggttca	XV	1.282	tttttgaag GAT TCT GGA
16	63	ATG CAT GAG gtaatatat	XVI	3.245	tgattatag GAT GAC AAT
17	98	TGG GTT ACG gtaagagtt	XVII	20.299	ttcattcag TGT TAT CTA
18	93	GGC CCC AAA gtaagtatg	XVIII	1.209	taattgcag GAG GAA AGC
19	109	CTG TTT GAG gtaggaatg	XIX	0.738	cttctgtag ATT CTT ATA
20	82	AAA CCC CTC gtatgtatg	XX	2.220	agattacag AGA TAC TAA
21	124	AAA CAG GAG gtaagctga	XXI	0.318	aattttcag GAG CTT GCT
22	193	CAT TCA AAG gtaagtttc	XXII	14.688	ttcacctag GTC ACT TTT
23	1059	GTG CTT GAG gtgatttta	XIII	0.982	tgtattaag ATA TCA AGG
24	179	GTG GAG AAG gtttgtcta	XXIV	1.148	tttggacag CCA TTC AAC
25	154	TCG GCT ACA gtaaggact	XXV	0.423	tctttacag CAT GAA CTG
26	181	TCC GAC TAG gtgagctgc	XXVI	4.039	aaattacag TTT GTG CAG
27	122	GCA GCG AAG gtaagtata	XXVII	0.450	cttaaatag AGC CCA GTG
28	108	AGA GAC ATA gtaagttac	XXVIII	12.124	ttttcccag GAG GAT AGC
29	160	CAC TCT CTG gtaagtttg	XXIX	3.193	atgatatag AAA TCA CAC
30	442	TTT TGA CAG gtactgata	XXX	10.928	ttattacag AAG TGT CAT
31	134	AAT CAC CAG gtgagttag	XXXI	8.713	cttttatag GCA GTA GAT
32	79	AAA TAT GAG gtatttaag	XXXII	1.909	tttccttag TAT TAC AGA
33	134	AAG GAA CAA gtaagtggt	XXXIII	7.964	ttaaaatag GTC TGG TTT
34	62	TGT TCT CAG gtgagtggc	XXXIV	35.939	tttttatag GAG TGG CAA
35	65	ATG AGG AAG gtaatttat	XXXV	26.429	ttcttacag GTT GCT TAG
36	109	GAA TTT GAG gtaggttac	XXXVI	>28.963	ctctccaag TCA CTG TGT
37	167	TGC AGT GAG gtaaaggga	XXXVII	83.886	cattgtag TCG TCC TCT
38	125	TGG AAC ATG gtcagtgg	XXXVIII	1.891	atgttttag TGT GCA TTT
39	33	ACA GCA AAG gtaagcatt	XXXIX	6.179	tcatttcag CCA CAG ATG
40	147	ATC TTG CCG gtaaatttg	XXXX	2.515	ttttggcag GTC CTG TTA
41	137	GAC CCC AAG gt	XXXXI	96.572	cctcattag ATC TTG GCA
42	118	CAA ACA GAG gtaatgtgt	XXXXII	3.088	ctgttgtag TTG CTG TGA
43	103	TCA AAC CAG gtactgttt	XXXXIII	15.997	ttcttgcag ACG TAT TTC
44	116	CGA TAG CAG gtaacctaa	XXXXIV	3.840	ccctatcag GAC GGA GTT
45	113	TTG TCC AAG gtaatttct	XXXXV	30.846	tattggcag CCA ATA GGA
46	141	CTA AGA ATA gtaagttca	XXXXVI	1.015	atttttag GAA CCC TTT
47	120	GAT ATT AAG gtacagaaa	XXXXVII	19.536	tttatatag GAG TTG ATC
48	153	AAC AGA TTG gtaagataa	XXXXVIII	65.358	ttttttcag GCC CTG GAG
49	169	TTG AGA GAG gtaagttat	XXXXIX	24.093	ccttttcag?GCT GTT GAA
50	90	ATG CAA GTG gtaagtgct	XXXXX	4.443	ctcctgcag AGT CCA TTG
51	178	ACC TTC CTG gtaagtaaa	XXXXXI	5.563	gaattccag CTC ATC AAG
52	63	CTC TCA TAG gtctgtcac	XXXXXII	5.176	ttcttacag CCA GCA ATA
53	156	CAG ACA CAG gtaattttc	XXXXXIII	7.441	gcattacag GAA GAT TGA
54	168	ACC CAG GCA gtaagtatg	XXXXXIV	16.043	ttcctaaag GTG AGA CTG
55	102	GTT CAC AAG gtaaacctg	XXXXXV	3.286	tcttctcag AAG GAC CAT
56	197	AAC ATA AGA gtgagtgcc	XXXXXVI	4.444	gtctcacag GCC ATC CAG
57	152	CGA CCA GAG gtaacactg	XXXXXVII	12.028	ttctcctag GTG CAT CAT
58	1148				
Total	9936			>716.138	

Example 6--Molec[redacted]Phylogenic Relationship of *hlrba* Protein[redacted]n Other WBWs

[00128] Phylogenic analysis of the WBW family reveals that the members can be grouped into two major families, as shown in Figure 12. One family is composed of proteins from C. elegans, D. melanogaster, H. sapiens, S. pombe, S. cerevisiae, A. thaliana, D. discoideum, and the other family contains proteins from H. sapiens, M. musculus, Dr. melanogaster, C. elegans, A. thaliana, B. taurus, L. major. These can be further sub-grouped into five distinct subfamilies, each of which may contains every species from the very ancient unicellular eukaryote to human. *Lrba* in human and murine, AKAP550 in fruit fly, F10F2.1 in C. elegans are orthologs as indicated previously, while NBEA and CG1332 are very close to *lrba* gene. *Lrba*, CHS1/beige and FAN belong to the same family. Despite the divergence of these species over several hundred million years, there is a high degree of sequence conservation in the BEACH domain, which may suggest an important role in the life of the cell concerning the BEACH domain.

Example 7--The human lrbaε alternative transcript has two in-frame ORF

[00129] The ORF prediction shows there are two in frame ORFs in the human *lrbaε* alternative transcript. One ORF encodes a 72 amino acid protein, another encodes a 2782 amino acid protein. A very conserved motif (p21 RAS motif IV(LLGVGGFD)) is missing from both proteins as a result of the disruption. Both ATGs are in the Kozak sequence and thus could serve as translation initiation sites. According to the translation scanning theory, the translation of the first ORF should not be a problem. There are three possibilities for the translation of the second ORF. The first possibility is leaking scanning, meaning that some ribosomes do not recognize the first ATG, but recognize the later ATG. However, there are four ATGs before the main ATG, and there is a long stem secondary structure between the two ORFs. Therefore, it is unlikely that the leaking model is the mechanism of translation. The second possibility is reading through translation, meaning that the translation machinery ignores the stop codon and reads through it. However, there are 10 stop codons between the two ORFs. Likewise, this is unlikely. A third possibility is that IRES (internal ribosome entry signal) translation is cap-independent. There is no homologous sequence between IRES, but they have complex secondary structure, such as long stem secondary structure. The RNA sequence between the two ORFs of human *lrbaε* can form a long stem structure, which could further make the leaking scanning or reading through impossible. Some mRNAs encoding pro-apoptic proteins, including Apaf-1 and DAP5 are also translated via an IRES

element. IRES-independent initiation is sometimes utilized during mitosis. The numberous mRNAs whose 5' UTR structures likely interfere with the 5' cap-dependent ribosome are good candidates for the presence of an IRES. However, the prediction of an IRES from only looking at the 5' UTR could be strengthened by a better understanding of the structural components that comprise these IRES elements.

Example 8--Identification of the five isoforms of the human *lrba* gene

[00130] Four isoforms that encode four different proteins are present in human *lrba* gene, among which three isoforms differ at C-terminal: h-*lrba*α has five WD repeats, h-*lrba*β lacks WD repeats, h-*lrba*δ lacks WD repeats and part of BEACH domain. The fourth isoform h-*lrba*γ has a YLLLQ additional sequence between BEACH domain and WD repeats. This insertion isoform also exists in murine *LRBA* gene, and the 15 bp nucleotide sequence insertion remains unchanged. All the isoforms are summarized as shown in Figure 13.

Table 3

Isoforms	Positions	Features	Implications	Pattern of alternative splicing*
1 α	There is one extra exon between Exon2 and Exon 3	Disrrupt the coding sequence of the *lrba* gene at the N-terminus	Bicistron may exist in eukaryotes. Ribosome Internal entry sequence.	Cassette
2 β	Poly(A) alternative splicing after Exon 48	There is a 312 bp Alu repeat sequence at the 5'UTR, splitting the BEACH domain at two third into two potential domains	1. The BEACH domain is not a minimum domain, could be actually composed of two domains. 2. The Alu sequence may regulate the translation of *LRBA* gene or other gene.	Multiple Polyadenylation Site
3 γ	15 bp insertion before Exon 51, just after BEACH domain and before WD repeats	The insertion encodes a YLLLQ peptide insertion into the *LRBA* protein.	Leucine (L) is a hydrophobic amino acid and may be involved in protein-protein interaction(as Leucine Zipper structure). That there are three consecutive Ls in a short sequence is unusual and	Retained intron

Isoforms	Pos[redacted]s	Features	Implications [redacted]	Pattern of alternative splicing*
			Y could be a potential target for phosphorilation.	
4 δ	Poly(A) alternative splicing after Exon 52	The isoform doesn't have WD repeats but BEACH domain	Although BEACH domain and WD repeats often stay together, they are separate domain and can exist and function separately.	Multiple Polyadenylation Site
5 ε	An additional exon at 5' end (Exon 5'-1) before Exon 1	Alternative promoter and transcription start site	*LRBA* may use different promoters to regulate the expression of *LRBA.*	Multiple Promoters

[00131] The *LRBA* gene and five isoforms of the *LRBA* gene are disclosed and characterized herein. Northern blot experiments show that expression of *lrba* is upregulated 2-4 fold following LPS stimulation of B cells and macrophages. A homology search of GENBANK reveals that *lrba* gene has othologues in *C. elegans, Drosophila*, mice and humans and paralogues in diverse species ranging from yeast to human. These genes define a new protein family that are designated the WBW gene family herein because the members share an evolutionarily conserved structure over a long protein sequence (around 1000 aa). The analysis of subcellular localization with a BEACH-WD-GFP fusion protein described herein provides the first direct evidence that the *lrba* member of the WBW family can physically associate with various vesicular compartments in cells. Furthermore, it is proposed that the *lrba* gene is also an AKAP, suggesting that WBW family proteins may have microtubule and PKA binding properties like AKAPs (Colledge, M. and J.D. Scott *Trends in Cell Biology*, 1999, 9:216). Studies of FAN suggest that WBW proteins can bind to cytoplasmic tails of activated receptors via their WD repeats (Adam-Klages, S. *et al. Cell*, 1996, 86:937).

[00132] The evidence suggests that WBW proteins are involved in intracellular vesicle trafficking. For example, the strikingly enlarged vesicles in *beige*/CHS cells occur in membrane-bound organelles. The CHS1/BG protein has a similar modular architecture to the VPS15 and Huntington proteins that are associated with the membrane fraction (Nagle, D.L. *et al. Nature Genetics*, 1996, 14:307) and the *lvsA* gene that is essential for cytokinesis (Kwak, E. *et al. Cell*, 1999, 10:4429)—a process that also involves fusion of intracellular

vesicles (Jantsch-[redacted]er, V. and M. Glotzer *Curr. Biol.*, 1999,[redacted]8; Heese, M. *et al. Curr. Opin. Plant Biol.*, 1998, 1:486). FAN may also be involved in vesicle trafficking since FAN-deficient mice, after cutaneous barrier disruption, have delayed kinetics of skin recovery that requires secretion of vesicles (Kreder, D. *et al. EMBO Journal*, 1999, 18:2472; Elias, P.M. *J. Invest. Dermatol.*, 1983, 80:44s). However, there is no direct evidence that these WBW proteins directly associate with vesicles. In contrast, others found unexpectedly by Western blot that the BG, LVSA, and DAKAP550 proteins are present in the cytosolic fraction of cells and not in the membrane fraction (Kwak, E. *et al. Cell*, 1999, 10:4429; Perou, C.M. *et al. Jour. Biol. Chem.*, 1997, 272:29790) or cytoskeleton (Han, J.D. *et al. Jour. Biol. Chem.*, 1997, 272:26611). This paradox can be explained by hypothesizing (without being limited by theory) that these proteins are not constitutively associated with vesicles, but rather associate with vesicles under certain conditions like LPS stimulation. This hypothesis agrees well with the observation that an LRBA-GFP fusion protein is located in the cytosol; however, it becomes associated with vesicles following activation of the cells by LPS stimulation. Confocal microscopy also shows this fusion protein co-localizes with the trans-Golgi and lysosomes. Immunoelectron microscopy further demonstrates that it is also localized to endoplasmic reticulum and the plasma membrane as well as the trans-Golgi complex and lysosomes. Therefore, it is established herein that the BEACH-WD-GFP fusion protein is associated with the vesicular system. This may be true for the intact LRBA protein as well as for other WBW proteins like CHS1/BG, LVSA, and FAN, since they share high homology with the region in mouse *lrba* that was used for the GFP fusion experiment. The activation-triggered vesicle trafficking hypothesis is further supported by the following: (1) BEACH domain contains a tyrosine phosphorylation site, (2) the WD repeats binding site of FAN contains a serine residue (Adam-Klages, S. *et al. Cell*, 1996, 86:937), it is possible that this serine is a target of serine kinases, as some experiments suggest that the WD repeats binding requires phosphorylation of the WD binding sites (Skowyra, D. *et al. Cell*, 1997, 91:209) and (3) MAPK was suggested to control the movement of lytic granules of NK cells (Wei, S. *et al. Jour. Exper. Med.*, 1998, 187:1753). Potentially, WBW protein functions are activated by tyrosine and/or serine/threonine kinases following stimulation by agents like LPS. Although the GFP fusion experiment previously described does not demonstrate that the BEACH domain and/or the WD repeats in LRBA directly associate with intracellular vesicles, it is proposed that the BEACH domain binds to vesicles while the WD repeat domains bind to a membrane-associated protein. It is proposed that because BEACH

domains and WD [redacted]ts exist separately in some proteins, they [redacted]e separate functions. For instance, the WD repeats of the FAN protein bind to the cytoplasmic tail of the TNFR55 receptor independent of the BEACH domain (Adam-Klages, S. *et al. Cell*, 1996, 86:937). It is worth noting that the FAN gene is made up almost entirely of the sequence in the highly conserved WBW structure (Figure 3), therefore other WBW-containing proteins may act like FAN and bind the cytoplasmic tails of TNFR55 or TNFR55-like receptors.

[00133] As indicated above, the *lrba* gene is a potential AKAP. The recently completed genomic sequence of *Drosophila* indicates that *lrba* has an orthologue in *Drosophila* (DAKAP550) that is capable of binding to protein kinase A (Han, J.D. *et al. Jour. Biol. Chem.*, 1997, 272:26611). The DAKAP550 gene is expressed in all tissues throughout development and is the principal A-kinase anchor protein in adult flies; it is enriched in secretory tissues such as neurons and salivary glands, and is found concentrated in the apical cytoplasm of some cells (Han, J.D. *et al. Jour. Biol. Chem.*, 1997, 272:26611), in agreement with the proposed function in secretion of *lrba*. Although the B1 and B2 RII binding sites of DAKAP550 are not present in mLRBA, hLRBA, and cLRBA, two sequences are disclosed that are very similar to the B1 and B2 RII binding sites in all *lrba* orthologues. The two sequences are predicted to form two adjacent amphipathic helices characteristic of PKA binding sites, satisfying the requirement of the hydrophobic interaction mechanism of RII peptide binding to the RII subunits of PKA revealed recently (Newlon, M.G. *et al. Nat. Struct. Biol.*, 1999, 6:222). Thus, *lrba* may serve as an AKAP that is involved in cAMP-mediated signaling secretory processes by translocating PKA to specific membrane sites. This translocation may require microtubule binding as suggested by the recent finding that another WBW protein, human CHS1, can associate with microtubules (Faigle, W. *et al. J. Cell Biol.*, 1998, 141:1121). Based on these findings, it is proposed a two-signal model for the function of the WBW protein family using the *lrba* gene as a protoype: LRBA is constitutively associated with PKA like other AKAPs and following LPS stimulation (signal one) the BEACH domain is phosphorylated. This enables the LRBA/PKA complex to bind to intracellular vesicles and tether vesicles to microtubules for transport to the plasma membrane. At the membrane, a second signal is required that generates cAMP. Binding of locally generated cAMP to the LRBA/PKA complex releases PKA, allowing it to phosphorylate cytoplasmic tails of activated receptors to enable binding of LRBA via its WD repeats. This final step would result in vesicle fusion with the plasma membrane (Figure 7). Many immune processes need a second signal such as in the case of co-stimulators. Without

being bound by t[redacted], it proposed that a first signal activate[redacted] immune cell to transport enough vesicles to the plasma membrane area that contact another cell. A second signal generated by the contact with the target cell produces cAMP that stimulates PKA activity resulting in membrane fusion of vesicles. Thus, LRBA and other WBW proteins may provide a means for eukaryotic cells to direct the fusion of membrane-bound vesicles in a polarized fashion, in coordination with signal transduction complexes at the plasma membrane as is required of many different effector cell types in the immune system (Stinchcombe, J.C. and G.M. Griffiths *Jour. Cell Biol.*, 1999, 147:1).

[00134] Increasing evidence suggests that all clinical symptoms of CHS/*beige* patients could be explained by a secretion malfunction. The cytolytic proteins (granzymes A/B and perforin) in CHS CTL are expressed normally, but are not secreted upon stimulation (Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122). Secretion of other enzymes are also defective in macrophages and neutrophils (Barak, Y. and E. Nir *American Journal of Pediatric Hematology-Oncology*, 1987, 9:42) as are the membrane deposition of class II molecules (Faigle, W. *et al. J. Cell Biol.*, 1998, 141:1121) and CTL-4 (Barrat, F.J. *et al. Proc. Natl. Acad. of Sci. USA*, 1999, 96:8645). However, there is a dispute over whether giant lysosomes in *beige*/CHS disease are a result of abnormalities in the fusion or fission of lysosomes (Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122; Barrat, F.J. *et al. Proc. Natl. Acad. of Sci. USA*, 1999, 96:8645; Perou, C.M. *et al. Jour. Biol. Chem.*, 1997, 272:29790; Cervero, C. *et al. Sangre*, 1994, 39:135; Barbosa, M.D. *et al. Nature*, 1996, 382:262; Menard, M. and K.M. Meyers *Blood*, 1988, 72:1726). How the secretion pathway is impaired is unclear. The characterization of the *lrba* gene and the model for its function, described herein, may provide a molecular explanation for these two major cellular dysfunctions of CHS/*beige*: giant vesicles and secretion malfunction. Vesicles may require association with the BEACH domain of CHS1 for fission and/or movement to the plasma membrane. After reaching the plasma membrane, they then require recognition of certain membrane proteins by the WD repeats to mediate fusion with the plasma membrane. This requires CHS1 proteins to be full-length for proper function since the WD repeats are at the COOH terminus. Thus, truncated beige/CHS protein molecules (or perhaps LRBA proteins) that lack the COOH terminal WD repeats would be expected to cause disease (Certain, S. *et al. Blood*, 2000, 95:979). The giant lysosomes in the affected cells may come from the failure of vesicle movement and/or fusion with the membrane. Similar disorders of *beige*/CHS have also been described in mink, cattle, cats, and killer whales. Given the structural similarity of

the WBW gene fa[redacted] it is proposed that the genetic mutations [redacted]ese species also involve other WBW genes. There are also other lysosomal trafficking mutants in mice with similar phenotypes to *beige* that may also involve mutation of other WBW gene family members.

[00135] In summary, the existence of a novel gene family, the WBW family, is demonstrated herein, which includes the *lrba* gene that: (1) is associated with the vesicular system, including the Golgi complex, lysosomes, endoplasmic reticulum, plasma membrane, and perinuclear ER, (2) is LPS inducible, (3) is an A kinase anchor protein (AKAP), and (4) has 5 different isoforms that differ in WD repeat number. These findings suggest an important role for *lrba* in coupling signal transduction and vesicle trafficking to enable polarized secretion and/or membrane deposition of immune effector molecules. This disclosure provides novel tools and methods that can be used to further the understanding of the mechanism of CHS and other related diseases as well as general immune cell function.

[00136] The cell membrane system not only delimits and protects cell and intracellular organelles, maintaining the essential differences between the cell interior and the environment, but also transports various molecules back and forth between the membrane-bound compartments in the cell, and between the cell and the environment through vesicle trafficking processes. These processes are critical for the correct biological functioning of a eukaryotic cell. A novel gene family, WBW, may play an essential role in vesicle trafficking has been identified in eukaryotic organisms from the very ancient unicellular organism Dictyostelium to human, but not in prokaryotes, which have no vesicle system (Wang, J.W. *et al. Journal of Immunology*, 2001, 166(7):4586-4595; Kwak, E. *et al. Mol. Biol. Cell*, 1999, 10(12):4429-4439; Adam-Klages, S. *et al. Cell*, 1996, 86(6):937-947; Barbosa, M.D. *et al. Nature*, 1996, 382(6588):262-265; Nagle, D.L. *et al. Nat. Genet.*, 1996, 14(3):307-311). The WBW proteins all have a highly conserved long WBW(WDL-BEACH-WD) structure composed of three domains at their C-termini (Wang, J. W. *et al. Journal of Immunology*, 2001, 166(7):4586-4595). WD domain is present in over two thousand proteins and is thought to be involved in protein-protein interaction (Smith, T.F. *et al. Trends Biochem. Sci.*, 1999, 24(5):181-185). The WD repeats of FAN bind to NSD motif of TNFR55 to mediate the activation of the plasma membrane-bound neutral sphingomyelinase, producing the secondary messenger ceramide to activate raf-1 and MAP kinases, leading to cell growth and inflammation responses (Adam-Klages, S. *et al. Cell*, 1996, 86(6):937-947). The function of the BEACH domain is unclear, it potentially has SH3 and SH2 binding sites and a tyrosine kinase phosphorylation site, and those sites may interact with multiple signal transduction

proteins (Wang, J. et al. *Journal of Immunology*, 2001, 1[redacted]):4586-4595). The WDL domain was first described in a previous publication, and its function also remains unknown (Wang, J. W. *et al. Journal of Immunology*, 2001, 166(7):4586-4595). However, the WBW structure is very conserved and the WBW structure of FAN represents most of its ORF, and thus it is reasonable to propose that the WBW structure has a similar function to that of FAN. Another interesting question is if WBW proteins are also AKAPs (A kinase anchor protein), as DAKAP550 and Neurobeachin have been experimentally proved to be AKAPs, which can direct protein kinase A to discrete intracellular locations, where PKA may be activated by the secondary messenger cAMP (Han, J.D. *et al. J. Biol. Chem.*, 1997, 272(42):26611-26619; Wang, X. *et al. J. Neurosci.*, 2000, 20(23):8551-8565). The subcellular localizations of the WBW proteins are not restricted to the plasma membrane, but are found in the Golgi complex, lysosomes, ER, perinuclear ER and clathrin-coated endocytosis pits (Wang, J. W. *et al. Journal of Immunology*, 2001, 166(7):4586-4595; Wang, X. *et al. J. Neurosci.*, 2000, 20(23):8551-8565), moreover are associated with microtubules (Faigle, W. *et al. J. Cell Biol.*, 1998, 141(5):1121-1134).

[00137] In the WBW family *chs1/beige* gene is the most extensively studied. The mutations of the gene can cause a generalized immunodeficiency in mice and humans with the impairment of NK cells, CTL, and granulocytes and often cause premature death in humans due to a second disease phase characterized by a lymphoproliferative syndrome, probably as a result of defective intracellular trafficking of vesicles (Spritz, R.A. *et al. J. Clin. Immunol.*, 1998, 18(2):97-105). For example, the deposition of some membrane proteins (HLA-DR) and antigen presentation are affected (Faigle, W. *et al. J. Cell Biol.*, 1998, 141(5):1121-1134). FAN has a role in TNF pathway by binding to a cytoplasmic motif upstream of the death domain of some TNF family receptors (TNFR55 and CD40) (Adam-Klages, S. *et al. Cell*, 1996, 86(6):937-947; Segui, B. *et al. J. Biol. Chem.*, 1999, 274(52):37251-37258). FAN knockout or FAN dominant-negative form can protect cell from apoptosis mediated by CD40 or TNF receptor (Segui, B. *et al. J. Clin. Invest.*, 2001, 108(1):143-151; Segui, B. *et al. J. Biol. Chem.*, 1999, 274(52):37251-37258). LvsA gene is essential for cytokinesis by possibly playing an important role in a membrane-processing pathway (Kwak, E. *et al. Mol. Biol. Cell*, 1999, 10(12):4429-4439). These studies suggest that the WBW proteins may play a role not only in vesicle trafficking, but also in some important cell processes like apoptosis and cell cycle.

[00138] However, the exact molecular mechanism of vesicle trafficking for the WBW proteins remains largely unclear. The mouse *lrba* (LPS-responsive beige-like PKA anchor gene) has its three isoforms, which differ at C-termini and have tissue-specific and development stage-specific expression pattern. *LRBA* gene is LPS inducible and can physically associate with various vesicular compartments in cells (Wang, J. W. *et al. Journal of Immunology*, 2001, 166(7):4586-4695). Described herein is the cloning, genomic structure and promoter analysis of the human *lrba* gene and its five isoforms. Its genomic locus consists of 58 exons and 57 introns, spinning over 700 K bps. Three isoforms (α, β, δ) differ at BEACH domain and WD repeats at their C-termini. The fourth isoform(γ) has a YLLLQ insertion sequence. The mRNA of the fifth isoform (δ) has two ORFs and a potential IRES for the translation of the second ORF. In the promoter region, there are four E2F binding sites and a CpG island, and surprisingly a potential p53 binding site was found in the promoter, suggesting that *lrba* gene may be involved in p53 mediated apoptosis or cell arrest, and E2F regulated cell cycle progress, and is regulated developmentally by CpG island. These results show that the *Lrba* gene is highly regulated at both the transcriptional and translational level, indicating that *lrba* gene may have a critical role in the life of the cell.

[00139] All patents, patent applications, provisional applications, publications, and nucleic acid and amino acid sequences associated with the GenBank accession numbers referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

[00140] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

1. A method for inhibiting the growth of tumors in a patient comprising administering an agent to the patient, wherein the agent suppresses lrba function in the patient.
2. The method according to claim 1, wherein the agent is a polynucleotide fragment of an lrba gene, or a variant thereof, or a polypeptide fragment of an lrba gene product, or a variant thereof.
3. The method according to claim 1, wherein the agent is a polynucleotide sequence encoding a fragment of the amino acid sequence set forth in Figure 1 or Figure 9, and wherein the amino acid sequence is at least five amino acids in length.
4. The method according to claim 1, wherein the agent is a polynucleotide comprising an lrba gene BEACH domain, an lrba gene WD domain, or both an lrba gene BEACH domain and an lrba gene WD domain, as set forth in Figure 9.
5. The method according to claim 4, wherein the WD domain is selected from the group consisting of the WDL domain, WD1 domain, WD2 domain, WD3 domain, WD4 domain, and WD5 domain, or a combination thereof.
6. The method according to claim 3, wherein the polynucleotide sequence is expressed within a tumor cell of the patient.
7. The method according to claim 3, wherein the polynucleotide is contained within a vector, and wherein the vector further comprises a promoter sequence operably linked to the polynucleotide.
8. The method according to claim 3, wherein the polynucleotide is contained within a recombinant cell, wherein said recombinant cell further comprises a promoter sequence operably linked to the polynucleotide.
9. The method according to claim 1, wherein the agent is an RNA sequence that interferes with expression of the lrba gene, and wherein the RNA sequence is selected from the group consisting of CCAGCAAAGGUCUUGGCUA (SEQ ID NO. 6), CAGUCGGGUUUGCGACUGG (SEQ ID NO. 7), UAGCCAAGACCUUUGCUGG (SEQ ID NO. 8), and GGGCACUCUUUCUGUCACC (SEQ ID NO. 9).

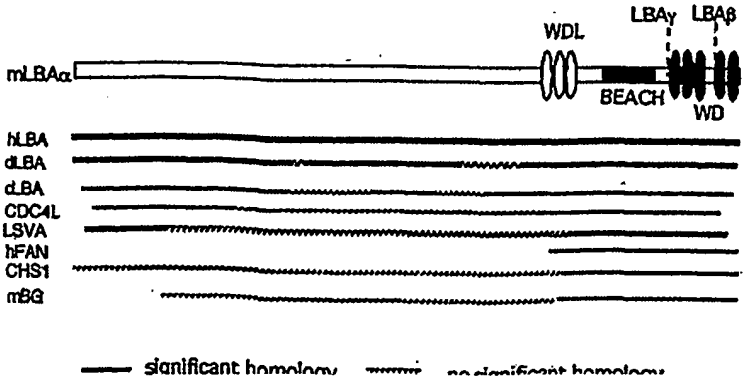
10. The method according to claim 1, wherein the agent is administered to a tumor site on the patient.
11. The method according to claim 1, wherein the patient is a mammal.
12. The method according to claim 1, wherein the patient is a human.
13. The method according to claim 1, wherein the patient is suffering from a tumor of a cancer type selected from the group consisting of breast, prostate, melanoma, chronic myelogenous leukemia, cervical cancer, adenocarcinoma, lymphoblastic leukemia, colorectal cancer, and lung carcinoma.
14. An isolated polynucleotide encoding the amino acid sequence set forth in Figure 1 or Figure 9, or a fragment or variant thereof.
15. The polynucleotide of claim 14, wherein said polynucleotide is selected from the group consisting of those set forth in GenBank accession numbers AF187731, AF188506, AF188507, and AF216648.
16. The polynucleotide of claim 14, wherein said polynucleotide is an lrba gene isoform selected from the group consisting of lrba-α, lrba-β, lrba-δ, lrba-γ, and lrba-ε, as set forth in Figure 9.
17. An isolated polynucleotide complementary to a polynucleotide encoding the amino acid sequence set forth in Figure 1 or Figure 9, or a fragment or variant thereof.
18. An isolated polypeptide comprising the amino acid sequence set forth in Figure 1 or Figure 9, or a fragment or variant thereof.
19. The polypeptide of claim 18, wherein said polypeptide is encoded by a polynucleotide sequence selected from the group consisting of those set forth in GenBank accession numbers AF187731, AF188506, AF188507, and AF216648.
20. The polypeptide of claim 18, wherein said polypeptide is encoded by an lrba gene isoform selected from the group consisting of lrba-α, lrba-β, lrba-δ, lrba-γ, and lrba-ε, as set forth in Figure 9.
21. A recombinant host transformed with a polynucleotide encoding the amino acid sequence set forth in Figure 1 or Figure 9, or a fragment or variant thereof, wherein said polynucleotide is operatively linked to a promoter sequence.

22. The recombinant host of claim 21, wherein said polynucleotide is selected from the group consisting of those set forth in GenBank accession numbers AF187731, AF188506, AF188507, and AF216648.
23. The recombinant host of claim 21, wherein said host is a prokaryotic cell.
24. The recombinant host of claim 21, wherein said host is a eukaryotic cell.
25. A vector comprising a polynucleotide encoding the amino acid sequence set forth in Figure 1 or Figure 9, or a fragment or variant thereof.
26. The vector of claim 25, wherein said vector is adenovirus.
27. The vector of claim 25, wherein said polynucleotide is selected from the group consisting of those set forth in GenBank accession numbers AF187731, AF188506, AF188507, and AF216648.
28. The vector of claim 25, wherein said vector further comprises a promoter sequence operatively linked to said polynucleotide.
29. An interfering RNA sequence selected from the group consisting of CCAGCAAAGGUCUUGGCUA (SEQ ID NO. 6), CAGUCGGGUUUGCGACUGG (SEQ ID NO. 7), UAGCCAAGACCUUUGCUGG (SEQ ID NO. 8), and GGGCACUCUUUCUGUCACC (SEQ ID NO. 9).

FIG. 1A

MASEDNRAPSRPPTGDDGGGGGKEETPTEGGALSLKPGLPIRGIRMKFAVLTG
LVEVGEVSNRDIVETVFNLLVGGQFDLEMNFIIQEGESIMCMVELLEKCDVTC
QAEVWSMFTAILKKSIRNLQVCTEVGLVEKVLGKIEKVDSMIADLLVDMLGVL
ASYNLTVRELKLFFSKLQGDKGQWPPHAGKLLSVLKHMPQKYGPDAFFNFPGK
SAAAIALPPIARWPYQNGFTFHTWLRMDPVNNINVDKDKPYLYCFRTSKGLGY
SAHFVGGCLIITSIKSKGKGFQHCVKFDFKPQKWYMVTIVHIYNRWKNSELRC
YVNGELASYGEITWFVNTSDTFDKCFLGSSETADANRVFCGQMTAVYLFSDAL
NAAQIFAIYQLGLGYKGTFKFKAESDLFLAEHHKLLLYDGKLSSAIAFMYNPR
ATDAQLCLESSPKDNPSIFVHSPHALMLQDVKAVLTHSIQSAMHSIGGVQVLF
PLFAQLDYKQYLSDEVDLTICTTLLAFIMELLKNSIAMQEQMLACKGFLVIGY
SLEKSSKSHVSRAVLELCLAFSKYLSNLQNGMPLLKQLCDHILLNPAVWIHTP
AKVQLMLYTYLSTEFIGTVNIYNTIRRVGTVLLIMHTLKYYYWAVNPQDRSGI
TPKGLDGPRPNQKEILSLRAFLLMFIKQLVMKDSGVKEDELQAILNYLLTMHE
DDNLMDVLQLLVALMAEHPNSMIPAFDQRNGLRVIYKLLASKSEGIRVQALKA
LGYFLKHLAPKRKAEVMLGHGLFSLLAERLMLQTNLITMTMYNVLFEILIEQI
CTQVIHKQHPDPDSTVKIQNPQILKVIATLLRNSPQCPESMEVRRAFLSDMIK
LFNNSRENRRSLLQCSVWQEWMLSLCYFNPKNSDEQKITEMVYAIFRILLYHA
VKYEWGGWRVWVDTLSITHSKVTFEIHKENLANIFREEQRKGDEETGPCSSSL
VPEGTGATRGVDVSVGSQHEDRKDSPISPHFTRNSDENSSIGRASSIDSASNT
ELQTHDMSSDEKKVERENQELLDQATVEETATNGAKDDLETSSDAAEPVTINS
NSLEPGKDTVTISEVSASISSPSEEDAAEMPELLEKSGVEEKEDDDYVELKVE
GSPTEEAGLPTELQGEGLVSAASGGREEPDMCGHGCEVQVEAPITKIHNDPET
TDSEDSRFPTVATAGSLATSSEVPVPQATVQSDSPEMLDGGMKATNLAGETES
VSDCADNVSEAPATSEQKITKLDVSSVASDTERFELKASTSTEAPQPQRHGLE
ISRQQEQTAQGTAPDAVDQQRRDSRSTMFRIPEFKWSQMHQRLLTDLLFSIET
DIQMWRSHSTKTVMDFVNSSDNVIEVHNLTHDISQVMDNMVMACGGILPLLSA
ATSATHELENIEPTQGLSIEASVDFLQRPISDIDVIPEASSLGFTEIEAEKNM
SSGGILRQCLRLVCAVAVRNCLECQQHSQLKARGDTAKSSKTIHSLIPMGKSA
AKSPVDIVTGGISSVRDLDRLPARTWTLIGLRAVVFRDIEDSKQAQFLALAVV
YFISVLMVSKYRDILEPQDERHSQSLKETSSDNGNASLPDAENTPAEESSLTL
SSVEESLEGTSCTRRRDSGLGEETASGLGSGLVSASPAAPLGVSAGPDAISEV
LCTLSLEVNKSQETRIDGGNELDRKVTPSVPVSKNVNVKDILRSLVNMPADGV
TVDPALLPPACLGALGDLSVDPPMQFRSFDRSVIIATKKSSVLPSALTTSAPS
SAVSVVSSVDPTHASDTGGESPGSRSPKCKTALSCKQLAPSHKTPAAHMSITE
RLEHALEKAAPLLREIFVDFAPFLSRTLLGSHGQELLIEGTSLVCMKSSSSVV
ELVMLLCSQEWQNSIQKNAGLAFIELVNEGRLLSQTMKDHLVRVANEAEFILS
RQRAEDIHRHAEFESLCAQYSADKREEEKMCDHLIRAAKYRDHVTATQLIQKI
INLLTDKHGAWGSSAVSRPREFWRLDYWEDDLRRRRRFVRNPLGSTHPEATLK
TAVEHAADEDILAKGKQSIKSQALGNQNSENEALLEGDDDTLSSVDEKDLENL
AGPVSLSTPAQLVAPSVVVKGTLSVTSSELYFEVDEEDPNFKKIDPKILAYTE
GLHGKWLFTEIRSIFSRRYLLQNTALEIFMANRVAVMFNFPDPATVKKVVNYL
PRVGVGTSFGLPQTRRISLATPRQLFKASNMTQRWQHREISNFEYLMFLNTIA
GRSYNDLNQYPVFPWVITNYESEELDLTLPSNFRDLSKPIGALNPKRAAFTAE
RFESWEDDQVPKFHYGTHYSTASFVLAWLLRIEPFTTYFLNLQGGKFDHADRT
FSSVSRAWRNSQRDTSDIKELIPEFYYLPEMFVNFNNYNLGVMDDGTVVSDVE
LPPWAKTSEEFVRINRLALESEFVSCQLHQWIDLIFGYKQQGPEAVRALNVFY
YLTYEGAVNLNSITDPVLREAVEAQIRSFGQTPSQLLIEPHPPRGSAMQASPL
MFTDQAQQDVIMVLKFPSNSPVTHVAANTQPGLAMPAVITVTANRLFAVNKWH
NLPAHQGAVQDQPYQLPVEIDPLIACGTGTHRRQVTDLLDQSIQVHSQCFVIT
SDNRYILVCGFWDKSFRVYSTDTGKLIQVVFGHWDVVTCLARSESYIGGNCYI
LSGSRDATLLLWYWNGKSSGIGDNPGGETATPRAILTGHDYEITCAAVCAELG
LVLSGSQEGPCLIHSMNGDLLRTLEGPENCLKPKLIQASREGHCVIFYENGCF
CTFSVNGKLQATVETDDHIRAIQLSRDGQYLLTGGDNGVVIVRQVSDLKQLFA
YPGCDAGIRAMALSFDQRCIISGMASGSIVLFYNDFNRWHHEYQTRY

FIG. 1B



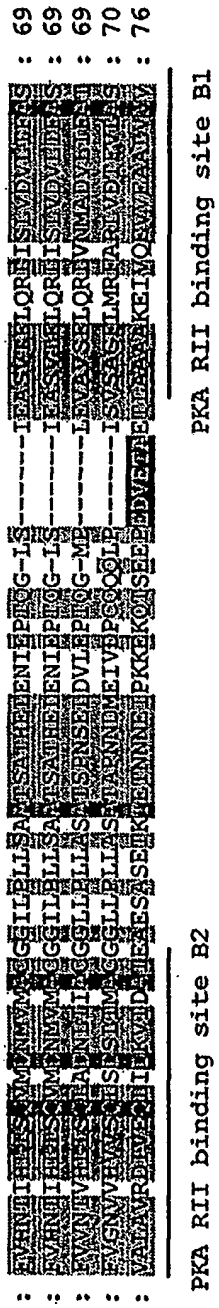

hLBA : 69
mLBA : 69
dLBA2 : 69
cLBA : 70
dLBA : 76
PKA RII binding site B2
PKA RII binding site B1

FIG. 2A

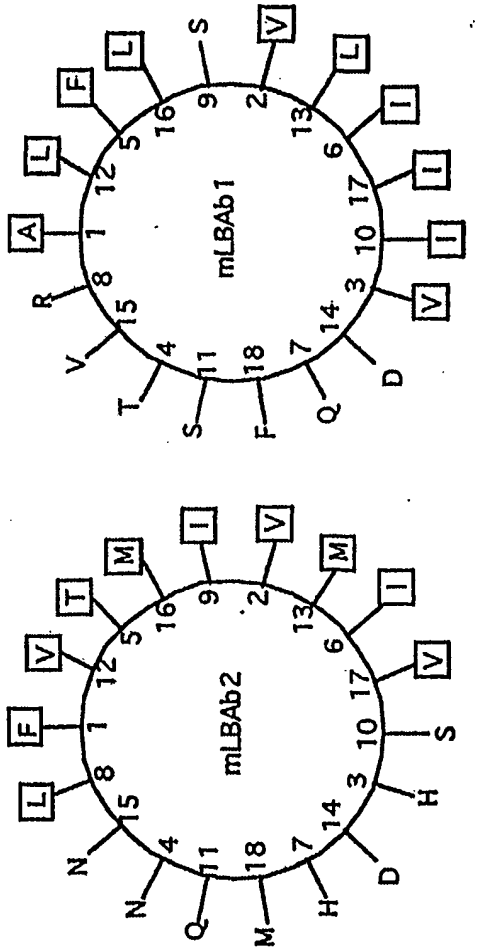

mLBAb1
mLBAb2

FIG. 2B

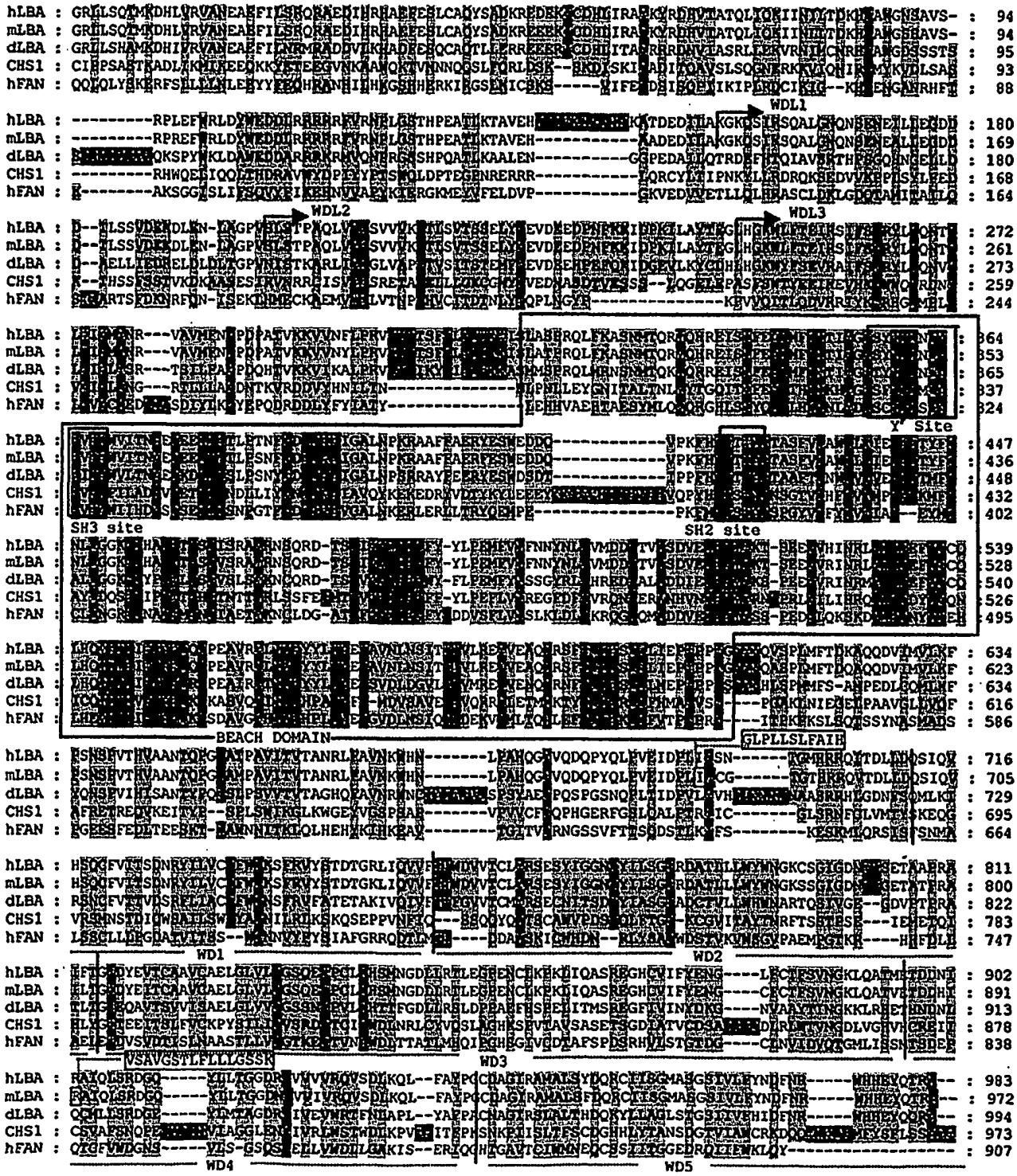

WDL1
WDL2
WDL3
Y Site
SH3 site
SH2 site
BEACH DOMAIN
WD1
WD2
WD3
WD4
WD5

FIG. 3

FIG. 4A

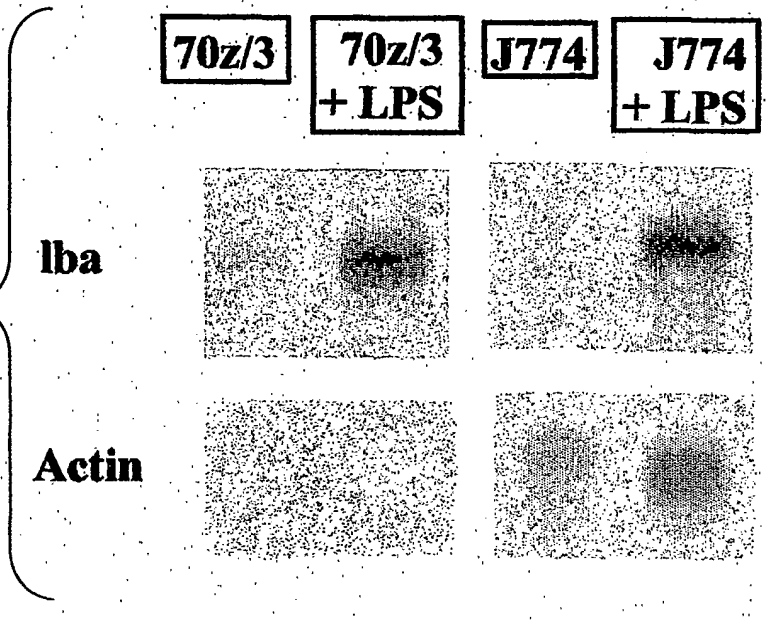


FIG. 4B

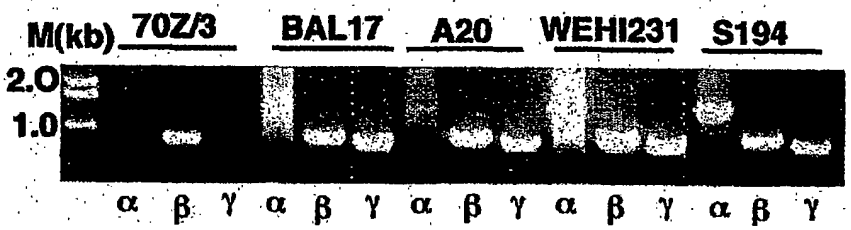


FIG. 4C

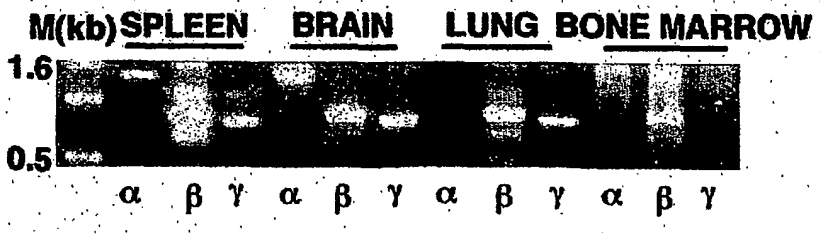


FIG. 5A FIG. 5B FIG. 5C

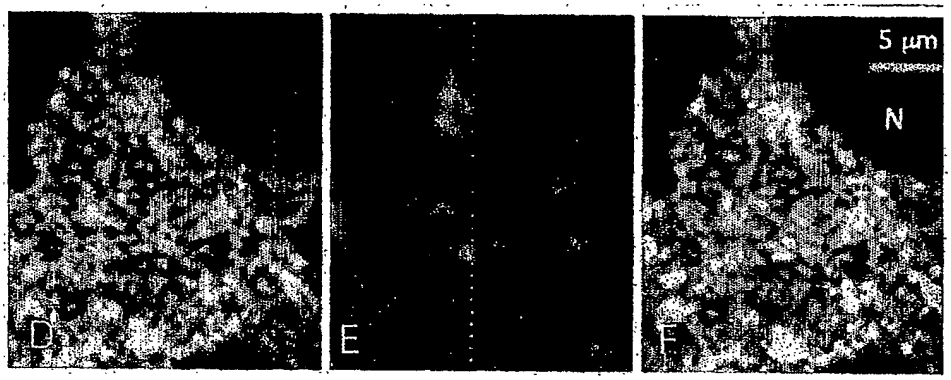


FIG. 5D FIG. 5E FIG. 5F

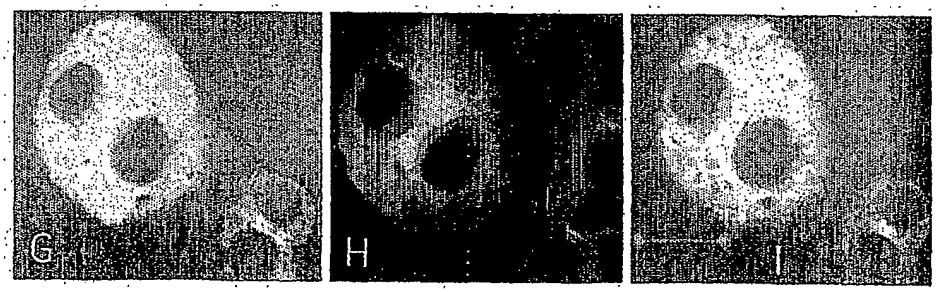


FIG. 5G FIG. 5H FIG. 5I

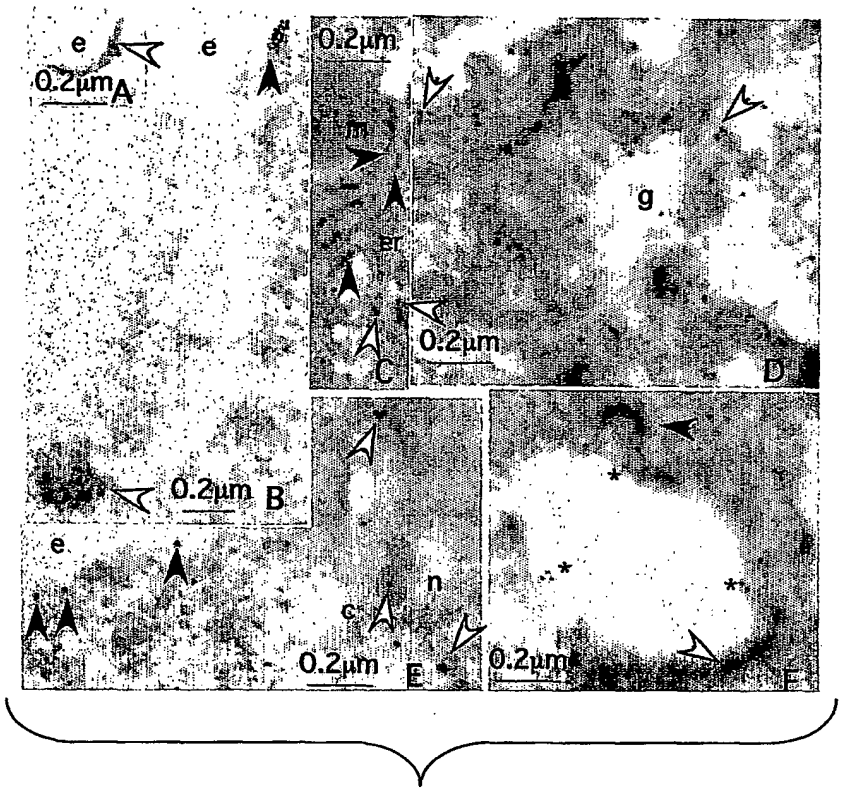

e
e
0.2μm
A
0.2μm
m
g
er
0.2μm
C
D
0.2μm
B
e
n
c
0.2μm
E
0.2μm
F

FIG. 6

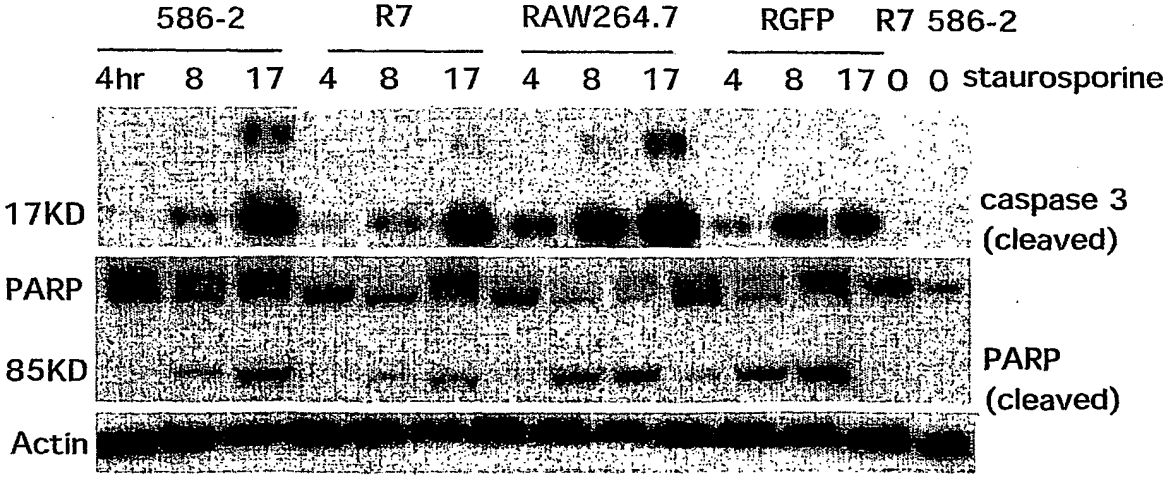

586-2
R7
RAW264.7
RGFP
R7 586-2
4hr 8 17 4 8 17 4 8 17 4 8 17 0 0 staurosporine
17KD
caspase 3
(cleaved)
PARP
85KD
PARP
(cleaved)
Actin

FIG. 8

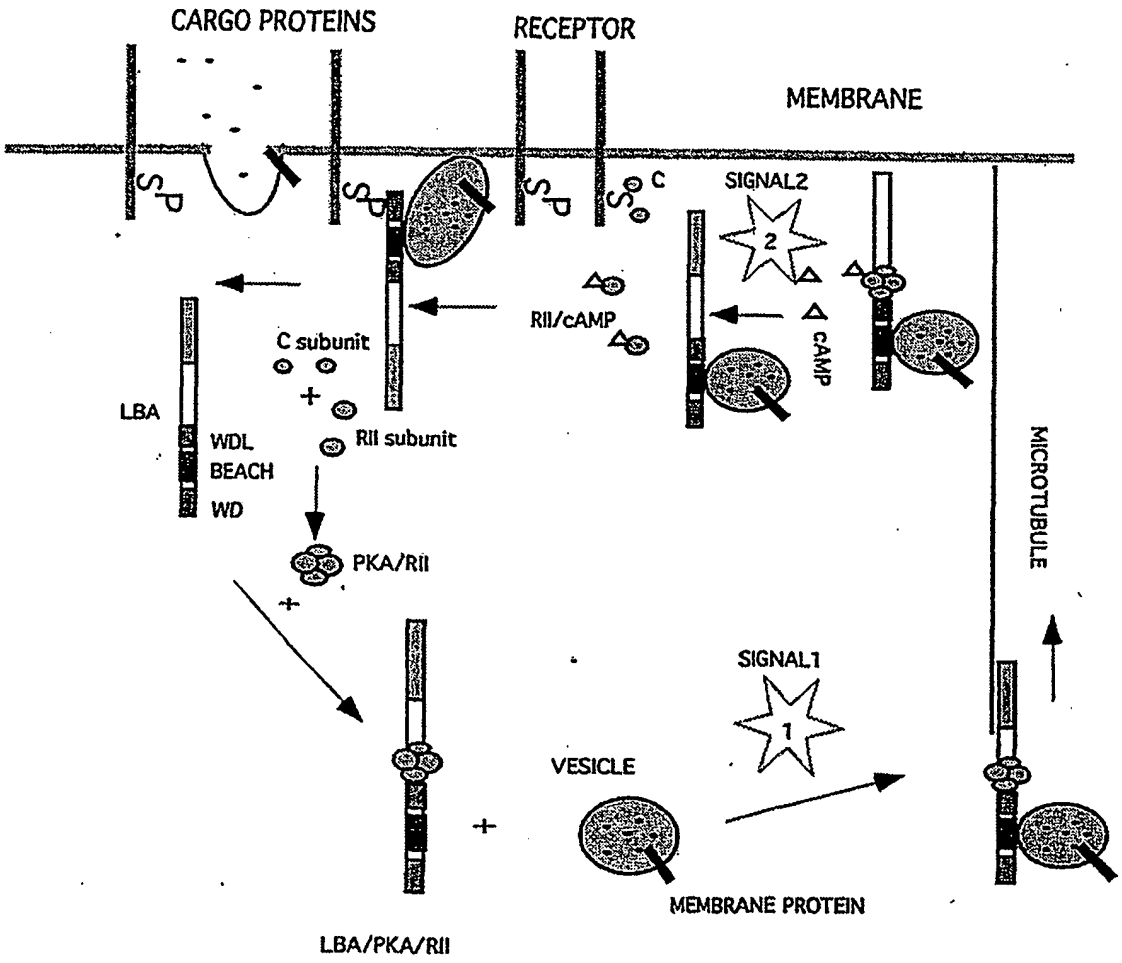

CARGO PROTEINS
RECEPTOR
MEMBRANE
SP
SP
SP
SP
C
SIGNAL2
2
RII/cAMP
cAMP
C subunit
+
LBA
WDL
BEACH
WD
RII subunit
PKA/RII
+
MICROTUBULE
SIGNAL1
1
VESICLE
+
MEMBRANE PROTEIN
LBA/PKA/RII

FIG. 7


G peptide
HSH domain
SET domain
WDL domain
BEACH domain
WD1
WD2
WD3
WD4
WD5

FIG. 9

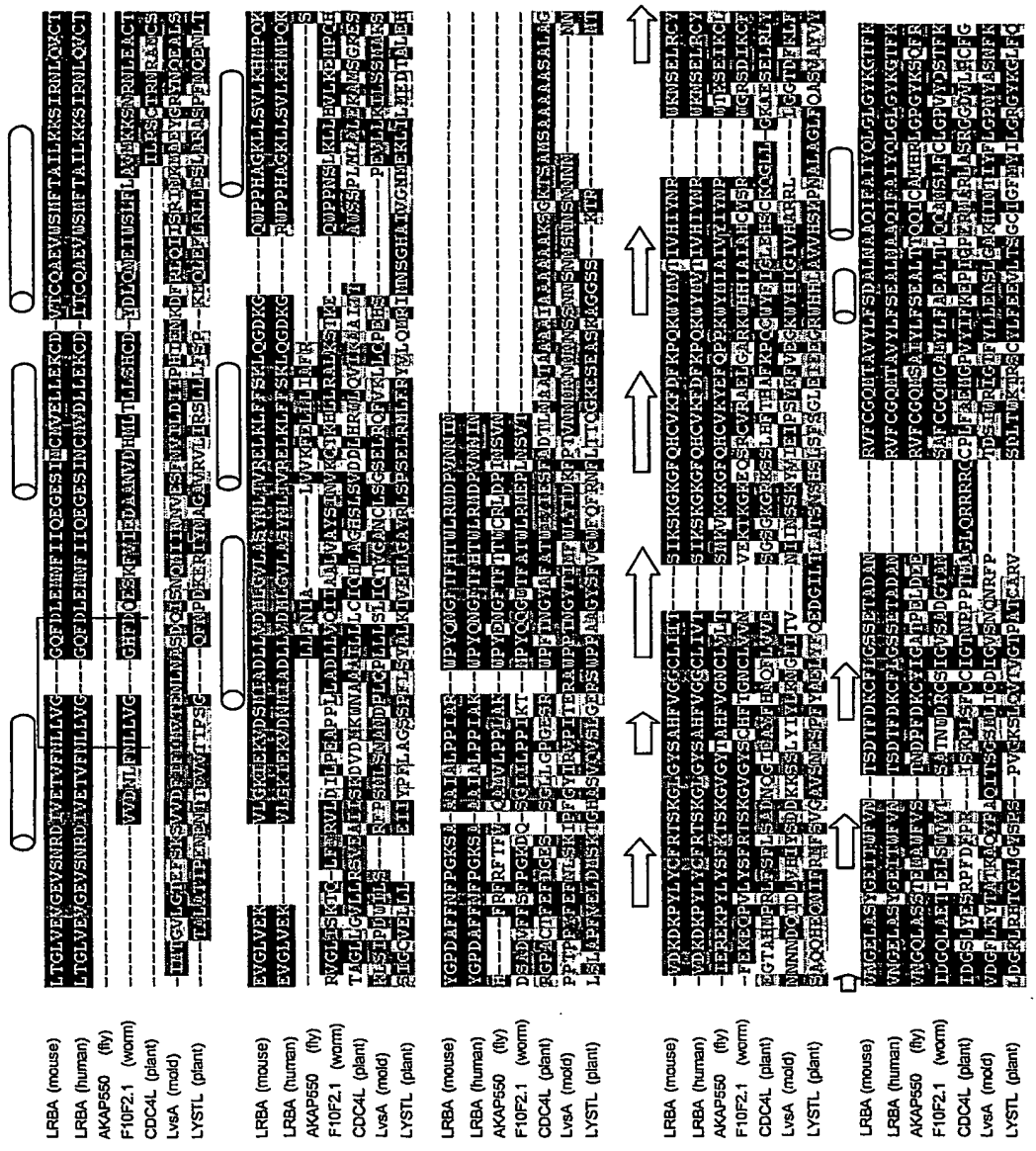

LRBA (mouse)
LRBA (human)
AKAP550 (fly)
F10F2.1 (worm)
CDC4L (plant)
LvsA (mold)
LYSTL (plant)

FIG. 10

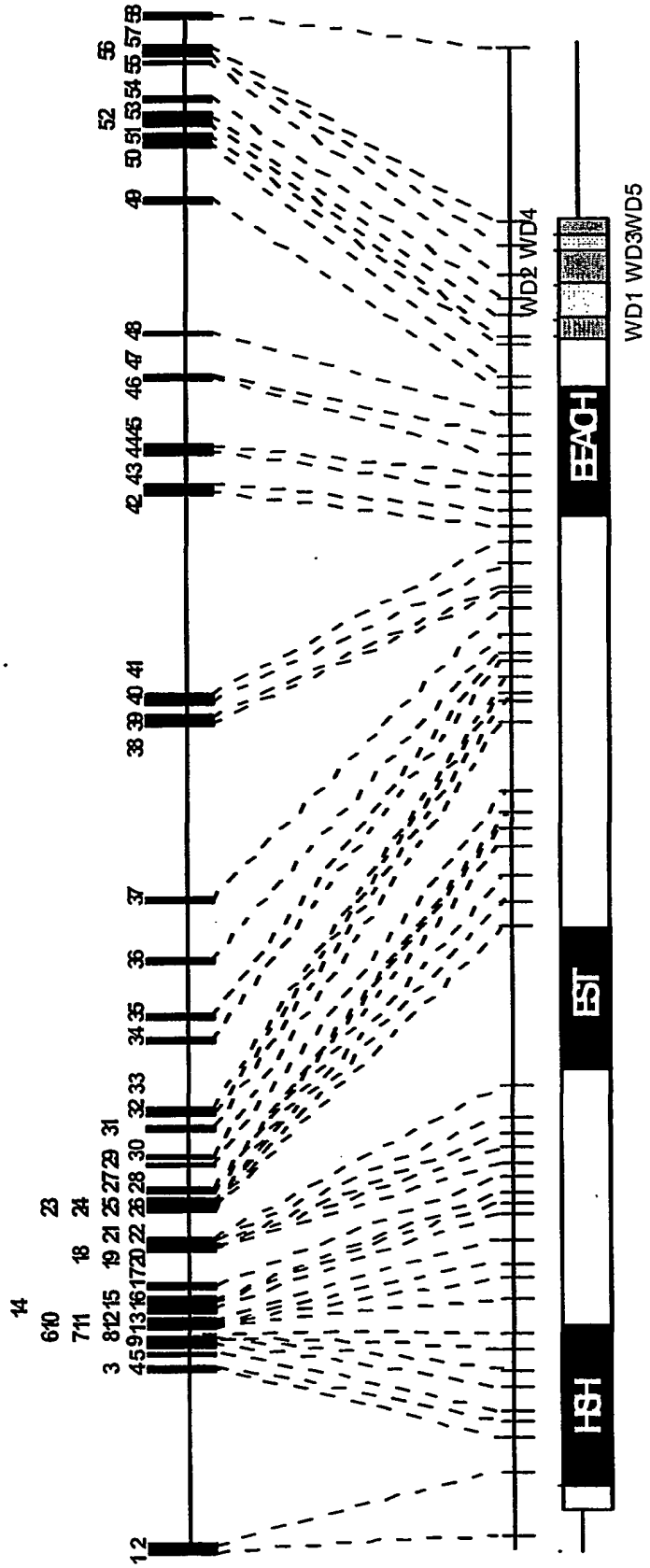


FIG. 11

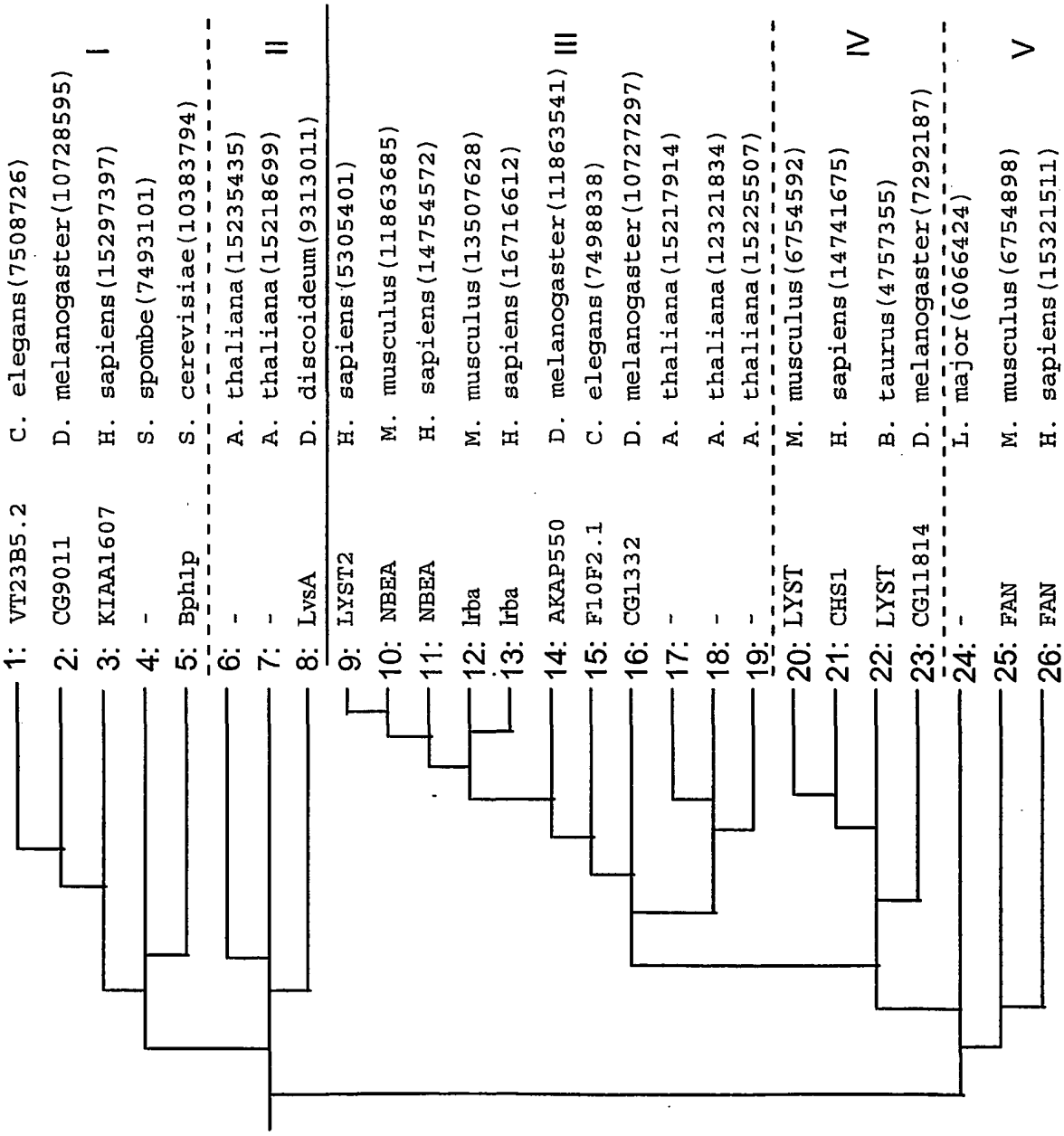

1: VT23B5.2 C. elegans(7508726)
2: CG9011 D. melanogaster(10728595)
3: KIAA1607 H. sapiens(15297397)
4: - S. spombe(7493101)
5: Bph1p S. cerevisiae(10383794)
6: - A. thaliana(15235435)
7: - A. thaliana(15218699)
8: LvsA D. discoideum(9313011)
9: LYST2 H. sapiens(5305401)
10: NBEA M. musculus(11863685)
11: NBEA H. sapiens(14754572)
12: Irba M. musculus(13507628)
13: Irba H. sapiens(16716612)
14: AKAP550 D. melanogaster(11863541)
15: F10F2.1 C. elegans(7498838)
16: CG1332 D. melanogaster(10727297)
17: - A. thaliana(15217914)
18: - A. thaliana(12321834)
19: - A. thaliana(15225507)
20: LYST M. musculus(6754592)
21: CHS1 H. sapiens(14741675)
22: LYST B. taurus(4757355)
23: CG11814 D. melanogaster(7292187)
24: - L. major(6066424)
25: FAN M. musculus(6754898)
26: FAN H. sapiens(15321511)
I
II
III
IV
V

FIG. 12

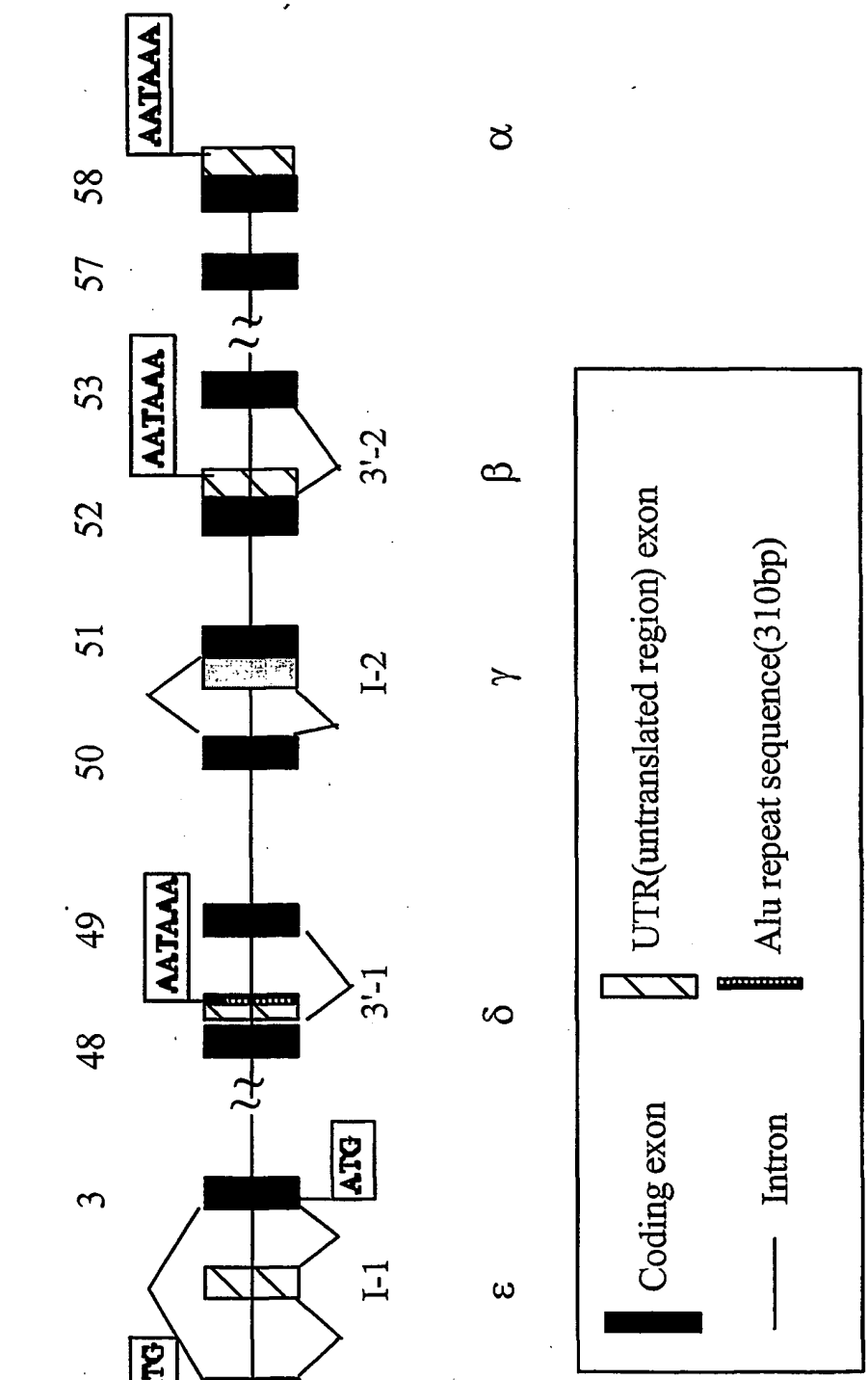

main exons
1
2
3
48
49
50
51
52
53
57
58
ATG
ATG
AATAAA
AATAAA
AATAAA
5'-1
I-1
I-2
3'-1
3'-2
alternative exons
Isoforms
ε
δ
γ
β
α
Coding exon
UTR(untranslated region) exon
Intron
Alu repeat sequence(310bp)

FIG. 13

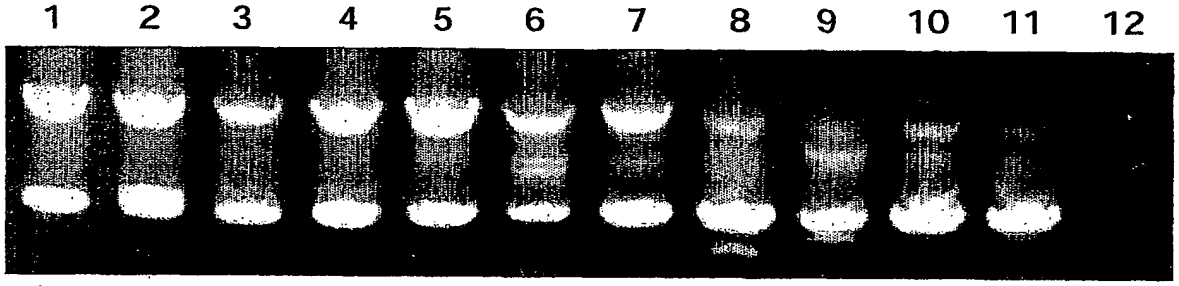
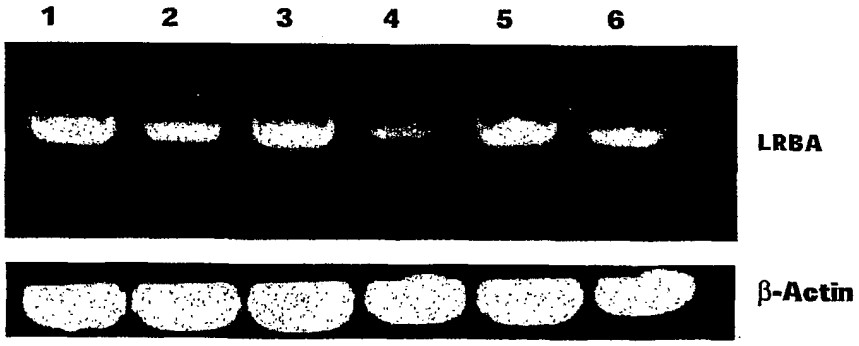


FIG. 14

FIG. 18A

FIG. 18B



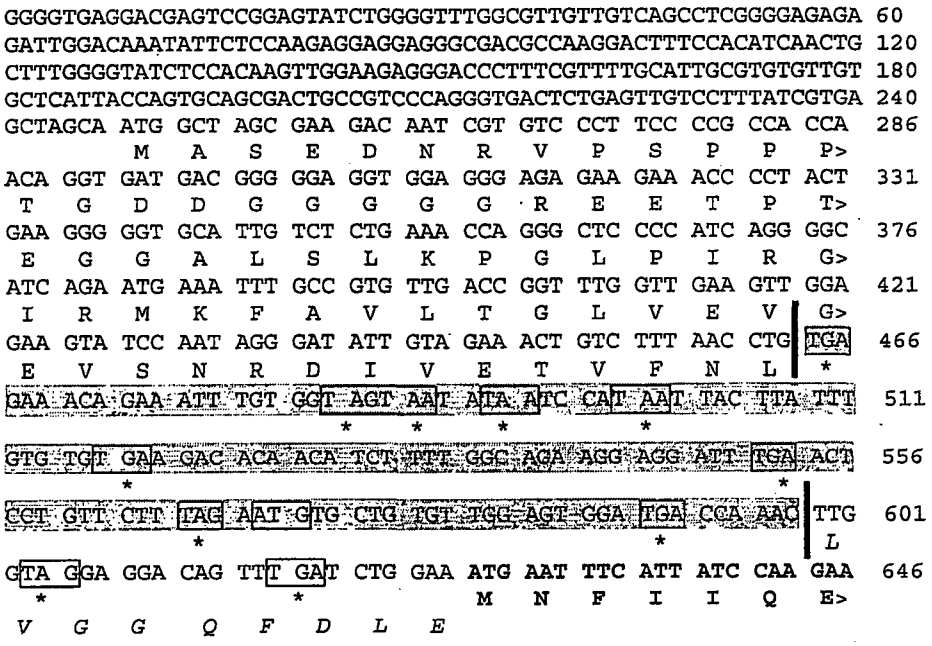

GGGGTGAGGACGAGTCCGGAGTATCTGGGGTTTGGCGTTGTTGTCAGCCTCGGGGAGAGA 60
GATTGGACAAATATTCTCCAAGAGGAGGAGGGCGACGCCAAGGACTTTCCACATCAACTG 120
CTTTGGGGTATCTCCACAAGTTGGAAGAGGGACCCTTTCGTTTTGCATTGCGTGTGTTGT 180
GCTCATTACCAGTGCAGCGACTGCCGTCCCAGGGTGACTCTGAGTTGTCCTTTATCGTGA 240
GCTAGCA ATG GCT AGC GAA GAC AAT CGT GTC CCT TCC CCG CCA CCA 286
M A S E D N R V P S P P P>
ACA GGT GAT GAC GGG GGA GGT GGA GGG AGA GAA GAA ACC CCT ACT 331
T G D D G G G G G R E E T P T>
GAA GGG GGT GCA TTG TCT CTG AAA CCA GGG CTC CCC ATC AGG GGC 376
E G G A L S L K P G L P I R G>
ATC AGA ATG AAA TTT GCC GTG TTG ACC GGT TTG GTT GAA GTT GGA 421
I R M K F A V L T G L V E V G>
GAA GTA TCC AAT AGG GAT ATT GTA GAA ACT GTC TTT AAC CTG TGA 466
E V S N R D I V E T V F N L *
GAA ACA GAA ATT TGT GGT AGT AAT ATA ATC CAT AAT TAC TTA TTT 511
GTG TGT GAA GAC ACA ACA TCT TTT GGC AGA AGG AGG ATT TGA ACT 556
CCT GTT CTT TAG AAT GTG CTG TGT TGG AGT GGA TGA CCA AAC TTG 601
L
GTA GGA GGA CAG TTT GAT CTG GAA ATG AAT TTC ATT ATC CAA GAA 646
M N F I I Q E>
V G G Q F D L E

FIG. 15

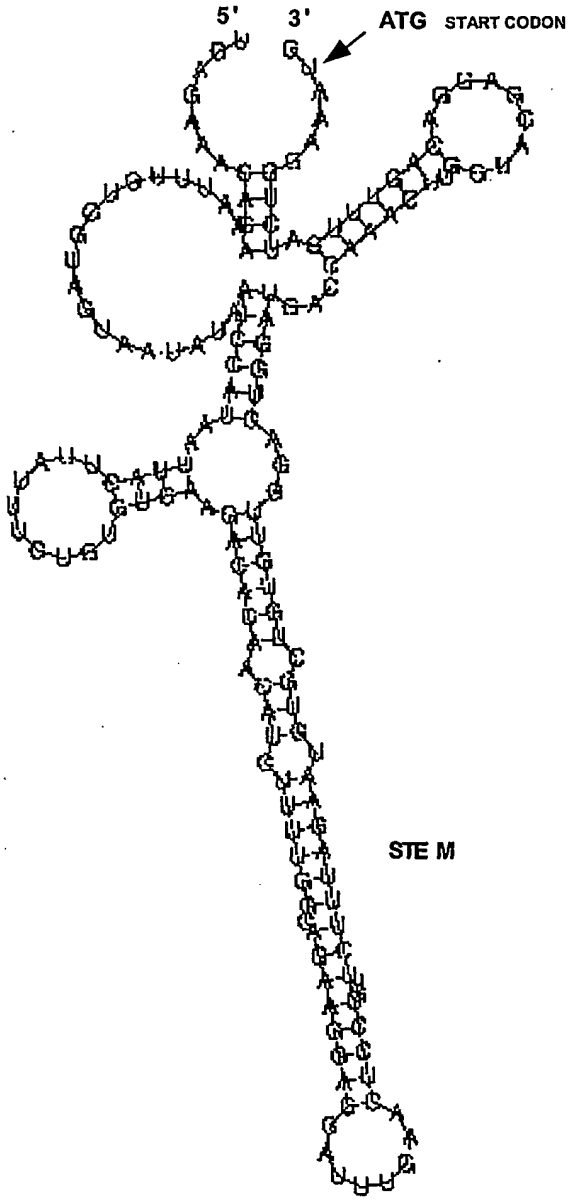


FIG. 16

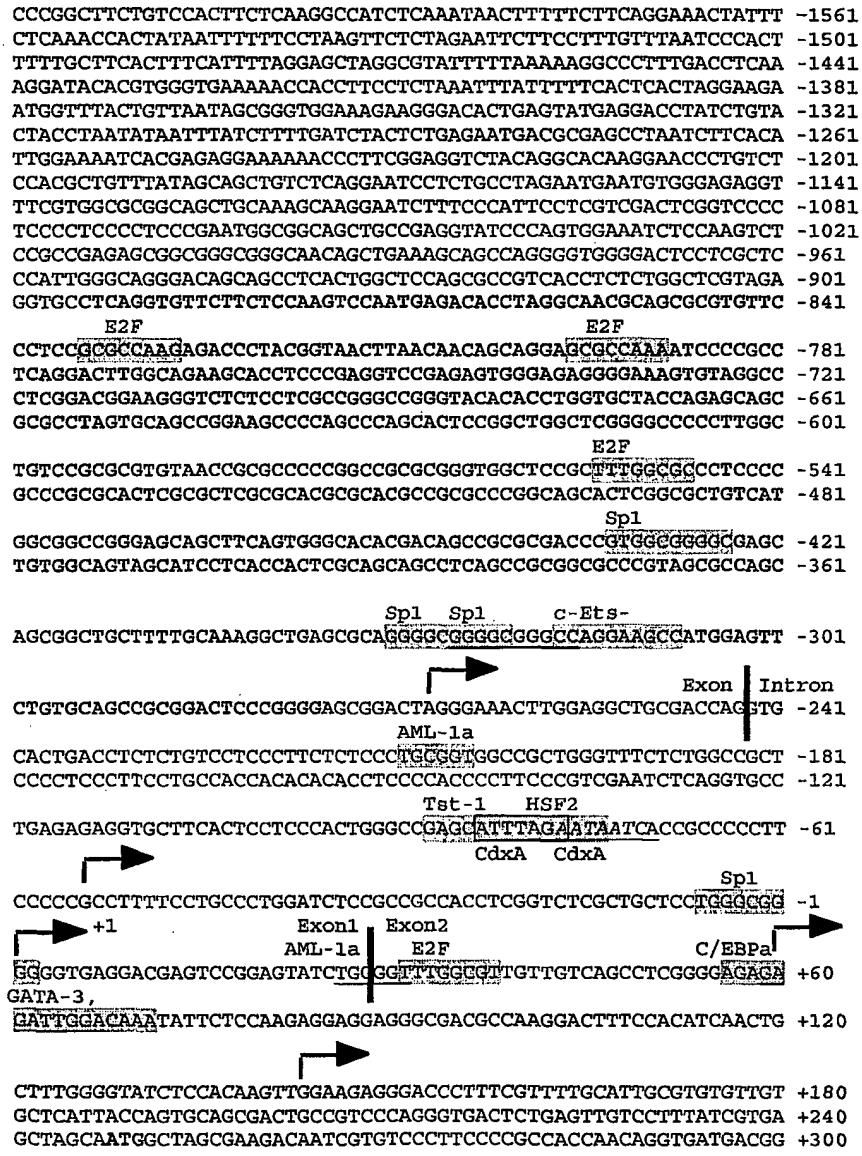

CCCGGCTTCTGTCCACTTCTCAAGGCCATCTCAAATAACTTTTTCTTCAGGAAACTATTT -1561
CTCAAACCACTATAATTTTTTCCTAAGTTCTCTAGAATTCTTCCTTTGTTTAATCCCACT -1501
TTTTGCTTCACTTTCATTTTAGGAGCTAGGCGTATTTTTAAAAAGGCCCTTTGACCTCAA -1441
AGGATACACGTGGGTGAAAAACCACCTTCCTCTAAATTTATTTTTCACTCACTAGGAAGA -1381
ATGGTTTACTGTTAATAGCGGGTGGAAAGAAGGGACACTGAGTATGAGGACCTATCTGTA -1321
CTACCTAATATAATTTATCTTTTGATCTACTCTGAGAATGACGCGAGCCTAATCTTCACA -1261
TTGGAAAATCACGAGAGGAAAAAACCCTTCGGAGGTCTACAGGCACAAGGAACCCTGTCT -1201
CCACGCTGTTTATAGCAGCTGTCTCAGGAATCCTCTGCCTAGAATGAATGTGGGAGAGGT -1141
TTCGTGGCGCGGCAGCTGCAAAGCAAGGAATCTTTCCCATTCCTCGTCGACTCGGTCCCC -1081
TCCCCTCCCCTCCCGAATGGCGGCAGCTGCCGAGGTATCCCAGTGGAAATCTCCAAGTCT -1021
CCGCCGAGAGCGGCGGGCGGGCAACAGCTGAAAGCAGCCAGGGGTGGGGACTCCTCGCTC -961
CCATTGGGCAGGGACAGCAGCCTCACTGGCTCCAGCGCCGTCACCTCTCTGGCTCGTAGA -901
GGTGCCTCAGGTGTTCTTCTCCAAGTCCAATGAGACACCTAGGCAACGCAGCGCGTGTTC -841
E2F
E2F
CCTCCGCGCCAAGAGACCCTACGGTAACTTAACAACAGCAGGAGCGCCAAAATCCCCGCC -781
TCAGGACTTGGCAGAAGCACCTCCCGAGGTCCGAGAGTGGGAGAGGGGAAAGTGTAGGCC -721
CTCGGACGGAAGGGTCTCTCCTCGCCGGGCCGGGTACACACCTGGTGCTACCAGAGCAGC -661
GCGCCTAGTGCAGCCGGAAGCCCCAGCCCAGCACTCCGGCTGGCTCGGGGCCCCCTTGGC -601
E2F
TGTCCGCGCGTGTAACCGCGCCCCCGGCCGCGCGGGTGGCTCCGCTTTGGCGCCCTCCCC -541
GCCCGCGCACTCGCGCTCGCGCACGCGCACGCCGCGCCCGGCAGCACTCGGCGCTGTCAT -481
Sp1
GGCGGCCGGGAGCAGCTTCAGTGGGCACACGACAGCCGCGCGACCCGTGGCGGGGCGAGC -421
TGTGGCAGTAGCATCCTCACCACTCGCAGCAGCCTCAGCCGCGGCGCCCGTAGCGCCAGC -361
Sp1 Sp1 c-Ets-
AGCGGCTGCTTTTGCAAAGGCTGAGCGCAGGGGCGGGGCGGGCCAGGAAGCCATGGAGTT -301
Exon Intron
CTGTGCAGCCGCGGACTCCCGGGGAGCGGACTAGGGAAACTTGGAGGCTGCGACCAGGTG -241
AML-1a
CACTGACCTCTCTGTCCTCCCTTCTCTCCCTGCGGTGGCCGCTGGGTTTCTCTGGCCGCT -181
CCCCTCCCTTCCTGCCACCACACACACCTCCCCACCCCTTCCCGTCGAATCTCAGGTGCC -121
Tst-1 HSF2
TGAGAGAGGTGCTTCACTCCTCCCACTGGGCCGAGCATTTAGAATAATCACCGCCCCCTT -61
CdxA CdxA
Sp1
CCCCCGCCTTTTCCTGCCCTGGATCTCCGCCGCCACCTCGGTCTCGCTGCTCCTGGGCGG -1
+1
Exon1 Exon2
AML-1a E2F
C/EBPa
GGGGTGAGGACGAGTCCGGAGTATCTGCGGTTTGGCGTTGTTGTCAGCCTCGGGGAGAGA +60
GATA-3,
GATTGGACAAATATTCTCCAAGAGGAGGAGGGCGACGCCAAGGACTTTCCACATCAACTG +120
CTTTGGGGTATCTCCACAAGTTGGAAGAGGGACCCTTTCGTTTTGCATTGCGTGTGTTGT +180
GCTCATTACCAGTGCAGCGACTGCCGTCCCAGGGTGACTCTGAGTTGTCCTTTATCGTGA +240
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FIG. 17

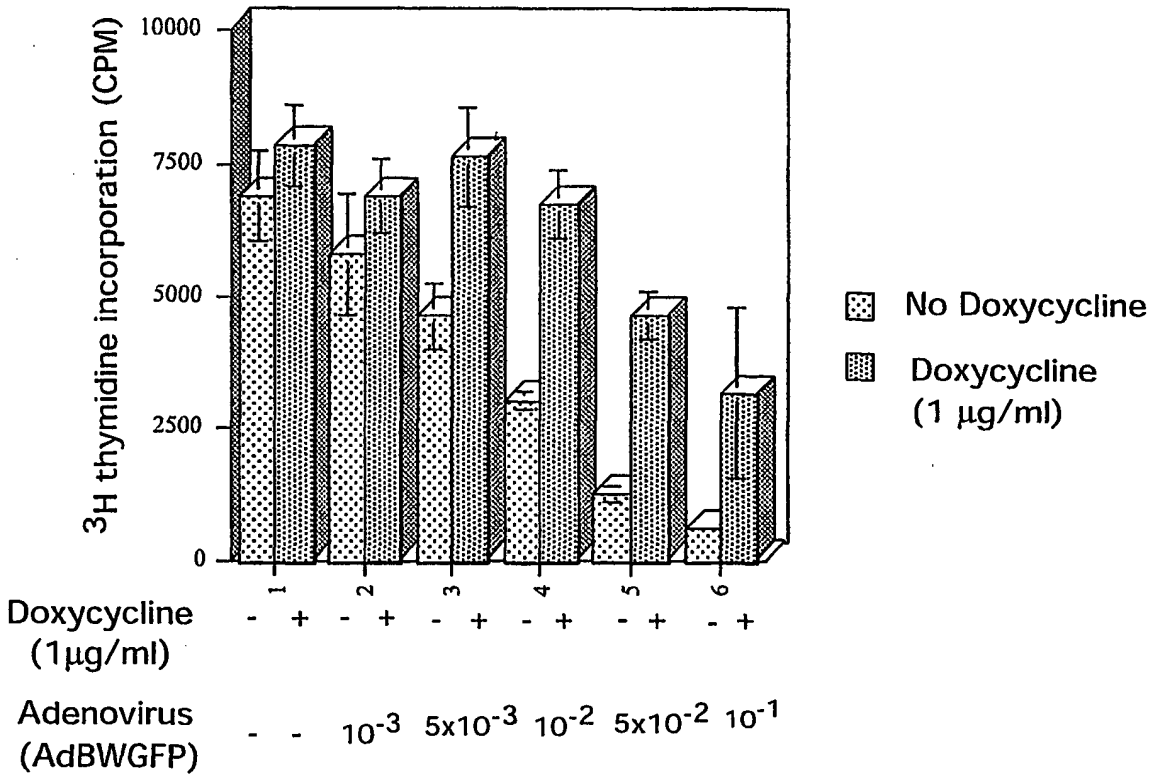

^{3}H thymidine incorporation (CPM)
10000
7500
5000
2500
0
1
2
3
4
5
6
Doxycycline (1µg/ml)
- + - + - + - + - + - +
Adenovirus (AdBWGFP)
- - 10^{-3} $5x10^{-3}$ 10^{-2} $5x10^{-2}$ 10^{-1}
No Doxycycline
Doxycycline (1 µg/ml)

FIG. 19

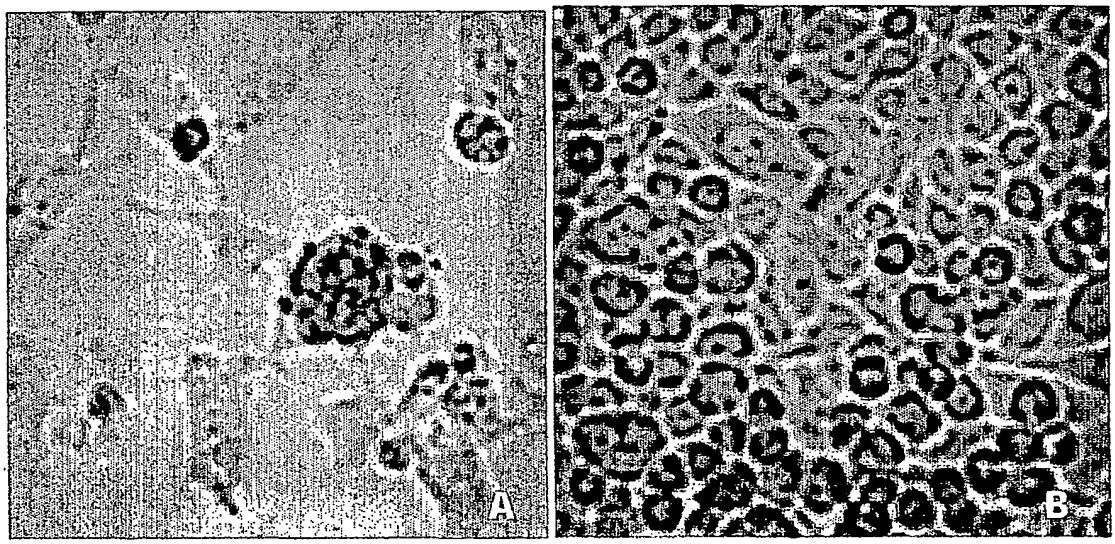


FIG. 20A FIG. 20B

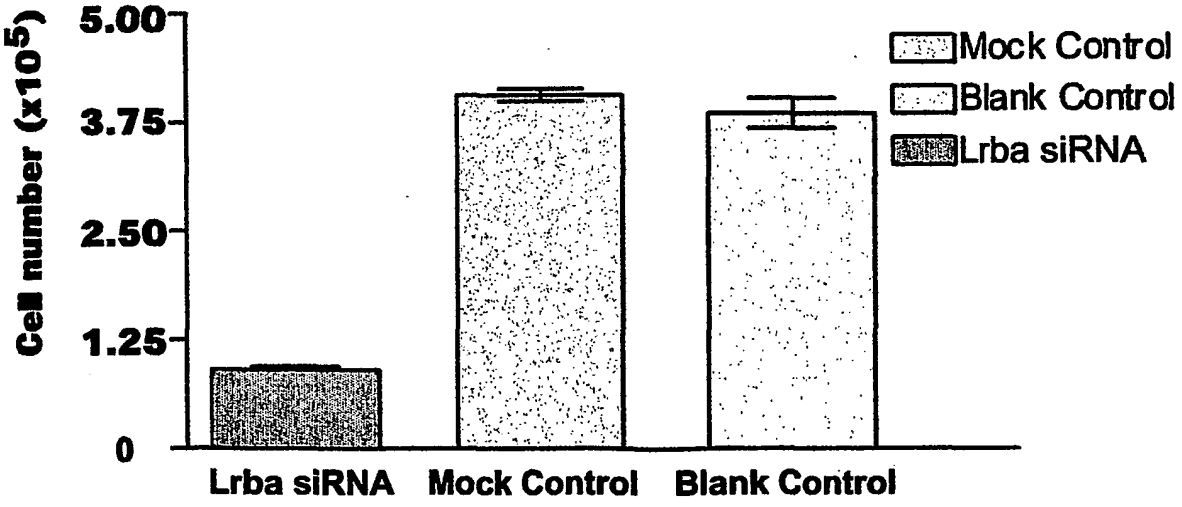


FIG. 20C

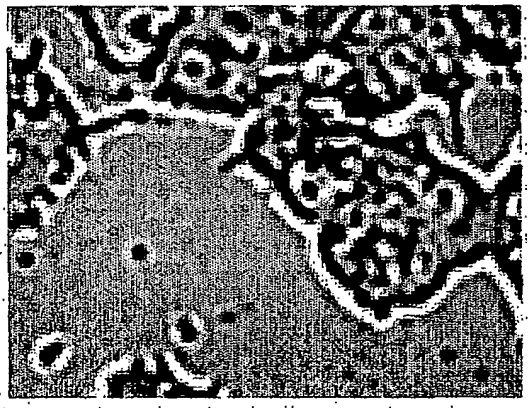


FIG. 21A

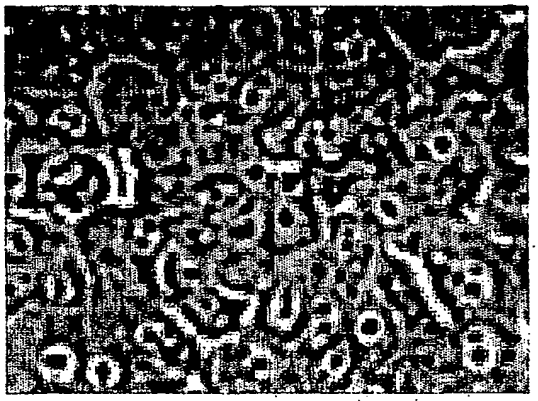


FIG. 21B

FIG. 21C

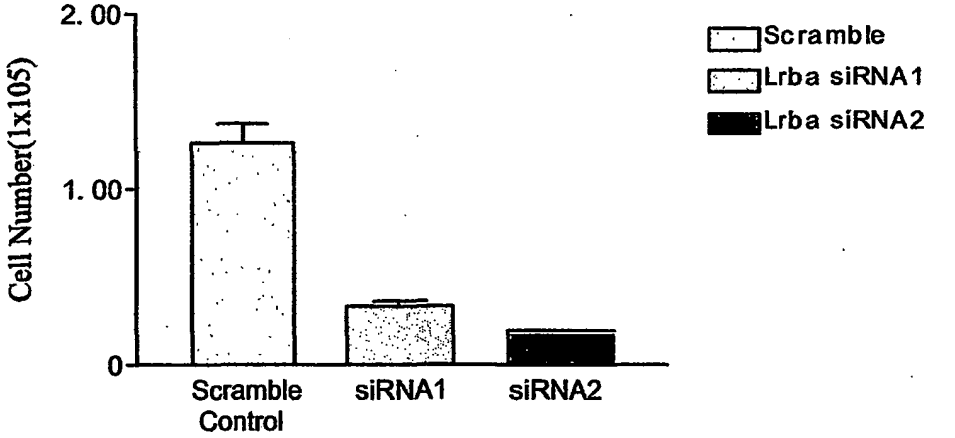
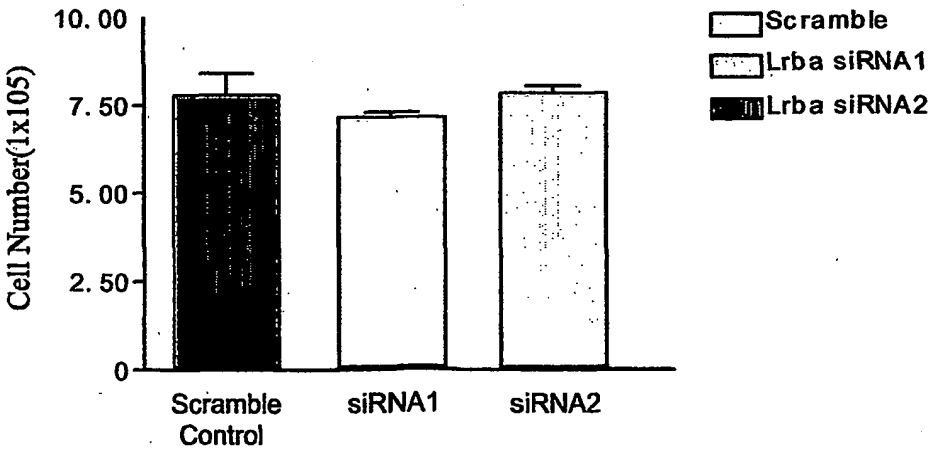


FIG. 21D



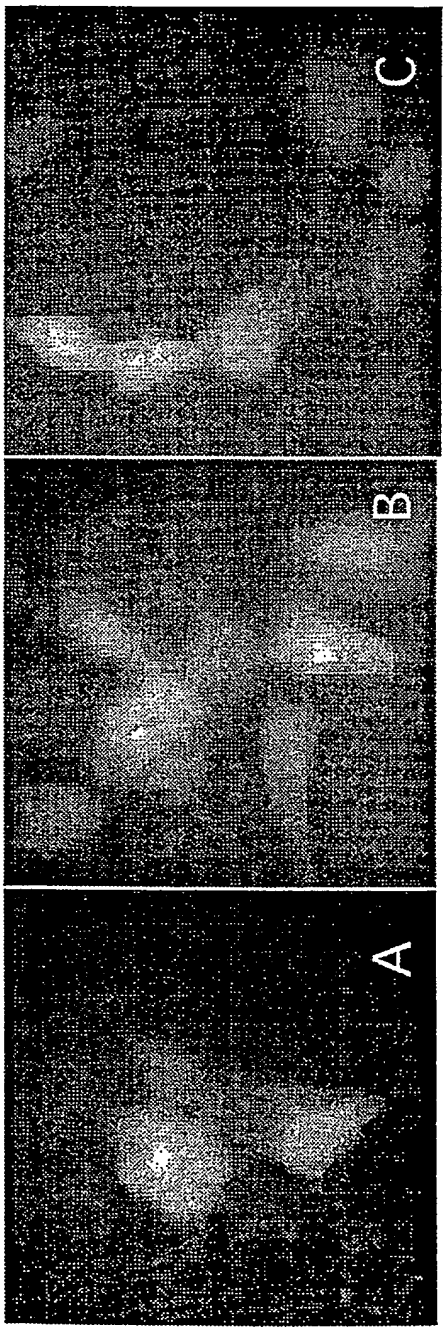


FIG. 22A FIG. 22B FIG. 22C

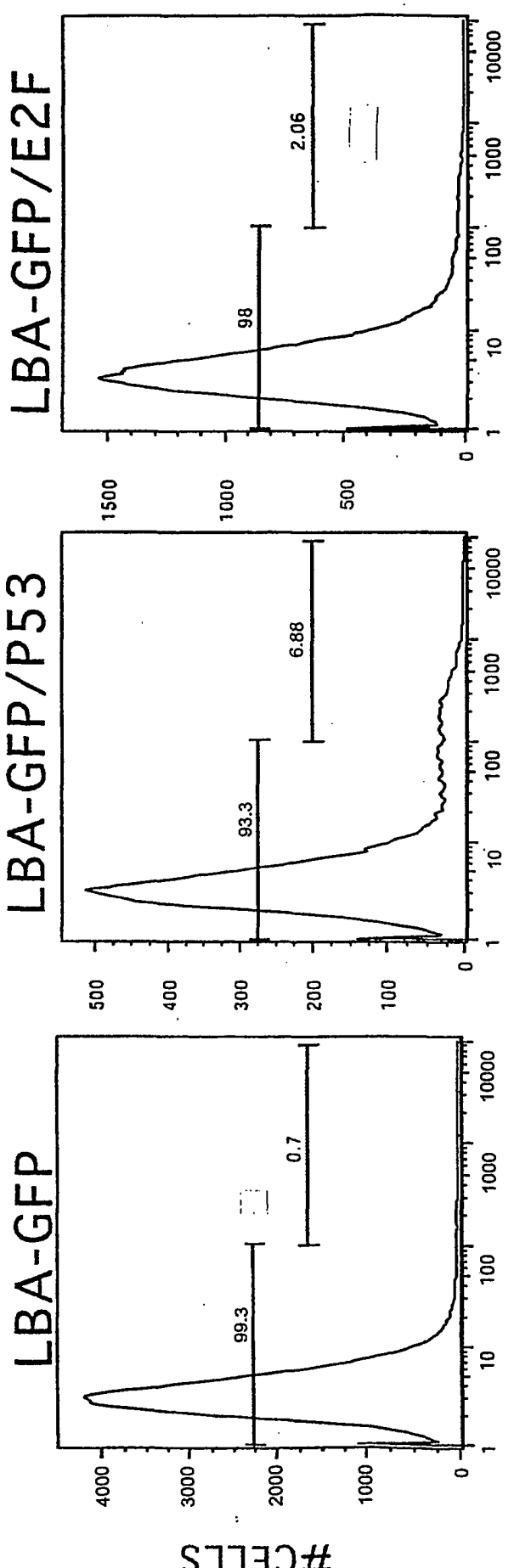


FIG. 22D

FIG. 22E

FIG. 22F

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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SOUTH FLORIDA** [US/US]; Division of Patents and Licensing, 4202 East Fowler Avenue, FAO 126, Tampa, FL 33620-7900 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KERR, William, G.** [US/US]; 4421 W. Watrous Avenue, Tampa, FL 33629 (US). **WANG, Jia-Wang** [CN/US]; 14011 Halstead Court 303, Tampa, FL 33613 (US).

(74) Agents: **LADWIG, Glenn, P.** et al.; Saliwanchik, Lloyd & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606 (US).

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(54) Title: LPS-RESPONSIVE CHS1/BEIGE-LIKE ANCHOR GENE AND THERAPEUTIC APPLICATIONS THEREOF

(57) Abstract: The present invention relates to a novel LPS-responsive and *Beige*-like Anchor gene (*lrba*), variants of the *lrba* gene, fragments of the *lrba* gene, and polypeptides encoded thereby. The subject invention also pertains to *lrba* interfering RNA, and uses thereof. In another aspect, the present invention also includes methods of inhibiting tumor growth in a patient by suppressing *lrba* function.

DESCRIPTION

LPS-RESPONSIVE CHS1/BEIGE-LIKE ANCHOR GENE AND THERAPEUTIC APPLICATIONS THEREOF

The subject invention was made with government support under a research project supported by the National Institutes of Health Grant Nos. RO1 DK54767, R21 AI44333, and PO1 NS27405. The government may have certain rights in this invention.

Cross-Reference to Related Application(s)

This application claims the benefit of provisional patent application Serial No. 60/280,107, filed April 2, 2001, which is hereby incorporated by reference in its entirety, including all nucleic acid sequences, amino acid sequences, figures, tables, and drawings.

Background of the Invention

[0001] Mutations in *chs1/beige* result in a deficiency in intracellular transport of vesicles that leads to a generalized immune deficiency in mouse and man. The function of NK cells, CTL, and granulocytes is impaired by these mutations indicating that polarized trafficking of vesicles is controlled by *chs1/beige* proteins. However, a molecular explanation for this defect has not been identified.

[0002] Lipopolysaccharide (LPS) is a potent inducer of maturation in B cells, monocytes, and dendritic cells that facilitates production of inflammatory cytokines, nitric oxide, and antigen presentation so that these cells can participate in the immune response to bacterial pathogens (Harris, M.R. *et al. Journal of Immunology*, 1984, 133:1202; Tobias, P.S. *et al. Progress in Clinical & Biol. Res.*, 1994, 388:31; Inazawa, M. *et al. Lymphokine Res.*, 1985, 4:343). In an attempt to identify genes involved in the maturation of immune cells, a gene-trapping strategy was developed to identify mammalian genes whose expression is altered by cellular stimuli (Kerr, W.G. *et al. Cold Spring Harbor Symposia on Quantitative Biology*, 1989, 54:767). Several novel LPS-responsive genes were successfully trapped (Kerr, W.G. *et al. Proc. Natl. Acad. of Sci. USA*, 1996, 93:3947), including the SHIP gene that plays a role in controlling the maturation and proliferation of B cells and

monocytes/macrophages *in vivo* (Huber, M. *et al. Prog. in Biophysics and Molecular Biol.*, 1999, 71:423; Ono, M. *et al. Nature*, 1996, 383:263; Ono, M. *et al. Cell*, 1997, 90:293).

[0003] *Chediak-Higashi* Syndrome (CHS^3) patients suffer from a systematic immune deficiency characterized by a severe immune defect, hypopigmentation, progressive neurologic dysfunction and a bleeding diathesis (Spritz, R.A. *Jour. of Clinical Immun.*, 1998, 18:97). Specific defects in immune cells include defects in T cell cytotoxicity (Abo, T. *et al. Jour. of Clinical Investigation*, 1982, 70:193; Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122), killing by NK cells (Haliotis, T. *et al. Jour. of Exper. Med.*, 1980, 151:1039), defective bactericidal activity and chemotaxis by granulocytes and monocytes (Clark, R.A. and H.R. Kimball *Jour. of Clinical Investigation*, 1971, 50:2645). CHS and *beige* lysosomes also exhibit compartmental missorting of proteins (Takeuchi, K. *et al. Jour. of Exper. Med.*, 1986, 163:665). Other studies have found that *beige* macrophages are defective for class II surface presentation (Faigle, W. *et al. J. Cell Biol.*, 1998, 141:1121; Lem, L. *et al. Jour. of Immun.*, 1999, 162:523) and that T cells in CHS patients are defective for CTLA4 surface expression (Barrat, F.J. *et al. Proc. Natl. Acad. of Sci. USA*, 1999, 96:8645). All cells in *beige* mice and CHS patients bear giant vesicles that cluster around the nucleus. Affected vesicles include lysosomes, platelet dense granules, endosomes, and cytolytic granules. These giant vesicles seem normal in several aspects except for their failure to release their contents, probably resulting from inability of the giant granules to mobilize and/or fuse with the membrane upon stimulation (Baetz, K. *et al. Jour. of Immun.*, 1995, 154:6122). However, despite these very provocative findings there still remains no direct evidence that BG(beige)/CHS1 proteins associate with intracellular vesicles and thus a molecular explanation for defective vesicle trafficking and protein missorting in these diseases is still sought.

Brief Summary of the Invention

[0004] The present invention relates to a novel LPS-responsive and *Beige*-like Anchor gene (*lrba*), its transcriptional/translational products, and the targeting of the *lrba* gene for the treatment of cancer. Thus, the present application is directed to the *lrba* gene, variants of the *lrba* gene, fragments of the *lrba* gene, corresponding polypeptides encoding by such nucleotides, and uses thereof. The mouse *lrba* gene is disclosed herein in Figure 1 (SEQ ID NO. 1) and the human *lrba* gene is disclosed herein in Figure 9 (SEQ ID NO. 2). The *lrba* gene is associated with the vesicular system, such as the Golgi complex, lysosomes,

endoplasmic reticu[redacted] plasma membrane and perinuclear ER, [redacted] plays an important role in coupling signal transduction and vesicle trafficking to enable polarized secretion and/or membrane deposition of immune effector molecules. In one aspect, the *lrba* variants of the subject invention include five isoforms of the *lrba* gene, including *lrba*-α, *lrba*-β, *lrba*-δ, *lrba*-γ, and *lrba*-ε. The sequences of the mouse *lrba* cDNAs have been deposited in GENBANK with the following GENBANK accession numbers: lrba-α: AF187731 (SEQ ID NO. 3), lrba-β: AF188506 (SEQ ID NO. 4), lrba-γ: AF188507 (SEQ ID NO. 5).

[0005] The subject invention also relates to cloning and expression vectors containing the *lrba* gene, and fragments and variants thereof, and cells transformed with such vectors.

[0006] In one aspect, the subject invention concerns *lrba* small interfering RNA (siRNA) sequences useful for the treatment of cancer. Preferably, the siRNA duplex is formed by annealing single-stranded RNA sequences (ssRNA) of 5'CCAGCAAAGGUCUUGGCUAdTdT3' (SEQ ID NO. 6) and 5'CAGUCGGGUUUGCGACUGGdTdT3' (SEQ ID NO. 7) from the *lrba* gene.

[0007] In a further aspect, the subject invention concerns methods of inhibiting the growth of tumors in a patient by suppressing *lrba* function. According to the method of the subject invention, suppression of *lrba* function can be carried out at various levels, including the levels of gene transcription, translation, expression, or post-expression. For example, suppression of *lrba* gene expression can be carried out using a variety of modalities known in the art for interfering with the production of a functional product of a target gene. For example, siRNA sequences, such as those described above, can be administered to a patient in need thereof. The siRNA can be produced and administered exogenously, or the siRNA can be inserted into an appropriate vector and the vector can be administered to the patient for production of the siRNA *in vivo*, for example.

[0008] The subject invention also provides methods of detecting the presence of *lrba* nucleic acids, transcriptional products, or polypeptides in samples suspected of containing *lrba* genes, transcriptional products, or polypeptides.

[0009] Another aspect of the subject invention provides kits for detecting the presence of *lrba* genes, *lrba* variants, *lrba* polypeptides, or *lrba* transcriptional products obtained from the polynucleotide sequences.

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